



## THE EFFECT OF ADMINISTRATION OF DIFFERENT LEVELS OF KEFIR MILK AS PROBIOTICS ON THE PERFORMANCE OF ARABI MALE LAMBS

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### ABSTRACT

This study was conducted at the College of Agriculture, the University of Basra from 24/10/2016 to 11/2/2017 to demonstrate the effect of supplementation of Kefir milk on performance and rumen parameters of male Arabi lambs. The study included 12 weaned male lambs at the age of 3 months with an average weight of (19.6±1.50 kg) randomly distributed to four treatments by three animals per treatment. All animals were fed barley (54%), soybeans (5%), bran wheat (3%), yellow corn (10), salts and vitamins (1%) (3% of body weight). Roughages introduced *ad libitum*. Straw was mixed with molasses and urea. Treated groups were control (no addition), the second, third and fourth group were given 40, 60, and 80 ml kefir/head/day respectively. The results revealed that the 3<sup>rd</sup> and 4<sup>th</sup> groups recorded significant differences in final body weight (33.40±3.38, 34.00±3.29 kg respectively) and total weight gain (13.80±1.49, 15.40±1.51 kg respectively) than the control group. Treatments showed a slight increase in feed intake and improve the efficiency of feed conversion compared with control group. The 3<sup>rd</sup> and 4<sup>th</sup> groups revealed significant differences in the average digestion coefficient of dry matter (70.24±1.27, 72.12±1.76%), protein (73.61±1.96, 75.45±1.89), fat (64.64±3.08, 68.51±3.20%) and fiber (63.51±1.15, 66.06±1.02%) compared with control group. Rumen pH value recorded by 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups during the second month (6.50±0.50, 6.73±0.68 and 6.16±1.02 respectively) were significantly higher than that of the control group. Total bacteria and cellulolytic bacteria counts of 3<sup>rd</sup> (65.23±6.44, 42.45±4.36 CFU/ml respectively) and 4<sup>th</sup> group (71.20±6.63, 44.76±5.95 CFU/ml respectively) were significantly higher than that of the control group. The 4<sup>th</sup> group showed higher lactic acid bacteria counts (30.00±5.21 CFU/ml) in comparison with that of the control group. The 3<sup>rd</sup> and 4<sup>th</sup> group showed a significant decrease in acetic acid concentration (69.19±0.29, 68.83±0.21% respectively) while the 4<sup>th</sup> group showed significant differences in propionic acid (19.00±0.17%) and butyric acid (8.00±0.13%) after 3h of feeding.

**KEYWORDS :** Kefir, Arabi, feeding, rumen parameters

### Introduction

Antibiotic was used for a very long time as either to eliminate diseases that infected animals or as growth promoters (fattening) and improve animal performance. Antibiotics have also positive influence through increasing nutrients digestion. However, it has a negative effect by establishing resistance strains that transmitted to human. Researchers investigated antibiotics alternatives such as probiotics which proved a significant effect on some productive and physiological traits (Ameta, 2011). The role of organisms of probiotics emerged from their ability to inhibit infected organisms and help to create new biological environment work on the determination of the special type of useful microorganisms to be an antibiotic to pathogens (Leticia *et al*, 2011). The idea behind Kefir emerged from probiotics function (FAO/WHO, 2011).

Ruminant nutritionists pay during past decades attention in developing a microbial system in the rumen in order to raise production efficiency through the use of antibiotics or growth promoters (Ahmed, 2012; Ali, 2011). Improving efficiency of rumen microorganism through increasing their effectiveness to degraded nutrients and provide direct sources of protein and energy (Homatouni *et al*, 2012).

Kefir is fermented milk produced by bacteria and yeast present in grains of this milk, when consumed in adequate amount influence health (FAO/WHO, 2011). Kefir has the high effectiveness of resistance to pathogens and improves animals' health (Yaman *et al*, 2006). There is intensive use of kefir in the food industry as well as anti-pathogenic factors and anti-fungi (Lopitz-Otsoa, 2006). The aims of this study were to determine the effect of supplementing different levels of kefir milk to male Arabi lambs on some production traits and rumen parameters.

### Materials and Methods

The present study was conducted at Animal Farm, College of Agriculture, University of Basra during the period from 24<sup>th</sup>/10/2016 to 11<sup>th</sup>/2/2017. The study included 12<sup>th</sup> weaned male lambs aged three months and weighted 19.60±1.50 Kg. Lambs distributed

equally and randomly on four treatments. The first treatment was control, lambs fed a basal concentration. Second, 3<sup>rd</sup> and 4<sup>th</sup> treatments fed basal ration as well as supplemented with 40 or 80 ml/lamb of kefir milk daily.

The feed was given as 3% of lambs' live body weight with *ad libitum* ammonia (3%) treated hay mixed with (10%) molasses. Concentrate ration was given twice daily at 7.00 in the morning and at 3.00 in the evening. Minerals blocks were provided *ad libitum*. All feed samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm sieve. Feed samples were subjected to proximate analysis following the standard methods of AOAC (1990). The DM, organic matter (OM), crude fiber (CF), ether extract (EE), and ash were determined according to the procedure outlined in AOAC (1990). Total N was determined using a Kjeldahl procedure with an automated, colorimetric quantification of ammonia in digested samples (AOAC, 1990) and multiplied by 6.25 to estimate crude protein (CP). The nitrogen-free extract (NFE) was calculated by differences. Concentrate composition and its chemical analysis are shown in the table (1).

Lambs were weighed individually at the beginning of the study (initial weight) and then biweekly until the end of the study in the morning after fasting (food and water) for 12-h. Amounts of feed offered and refused were recorded, and daily DM intake was calculated. As well as growth rate was calculated as follows: average daily gain (ADG, g/lamb/d) was calculated as the difference between the final BW and initial BW divided by the number of days on feed. Total weight gain (TWG, kg) was measured as the difference between final BW and initial BW. Growth rate (GR, %) = (final BW – initial BW) / (initial BW) × 100. The feed conversion ratio (FCR) was calculated as the ratio between DM intake and daily gain (g of DM intake/g of BW gain).

**Table (1) concentration composition and chemical components**

Feed	%
Barley	54

<b>Wheat Bran</b>	30
<b>Soya Bean Meal</b>	5
<b>Corn</b>	10
<b>Minerals &amp; Vitamins</b>	1
<b>Chemical Components</b>	
<b>Dry Matter</b>	89.72
<b>Crude Protein</b>	12.89
<b>Ether Extract</b>	3.33
<b>Crude Fiber</b>	7.20
<b>Nitrogen Free Extract (NFE)</b>	63.70
<b>Metabolic Energy (MJ/kg)</b>	11.85

\*metabolic energy was measured according to MAFE (1975)

Samples of rumen fluid were collected monthly from the beginning until the end of the study after 3 and 6 h of feeding. Rumen fluid samples pH was estimated by a digital pH meter (PW Philips 9909) immediately after collection of rumen samples. Total microorganisms, cellulolytic bacteria (*Ruminococcus flavefaciens*) and lactic acid bacteria were determined as shown by Baltaci and Ahmet (2016).

To determine nutrients digestibility rate each treated lamb assigned in individual digestibility cages during the last week of the study. Daily fecal collections were weighed and mixed thoroughly by hand and subsamples representing 10% of daily fecal production from each lamb were frozen at -5°C until being composited for the complete period collection. Representative samples of each daily collection feces were pre-dried in drying oven at 60°C to 70°C for 48 h and ground through 1 mm mill screen openings and were stored for further analysis. Samples were analyzed for DM, OM, CP, EE, CF, and ash contents according to AOAC (1990) methods, while NFE was calculated by differences.

Fatty acids concentration of rumen fluid was determined by GC (HIMADZU, Japan) as described by Luo *et al.* (2015). Statistical analysis was done within the SPSS (2013, version, 22) program.

**Results and Discussion**

**Body weight and growth rate**

Table (2) showed significant differences in the mean of body weight and growth rates due to different treatments. Lambs received 60 or 80 ml of kefir revealed higher (p<0.05) body weight and growth rates at 2<sup>nd</sup> and 3<sup>rd</sup> month of study in comparison with lambs of the control group. These results reflected that the addition of probiotics of single or multiple strains of bacteria significantly improved feed intake, feed conversion ratio, growth rates and live body weights of sheep, goats, and cattle (Stein *et al.*, 2006 and Casey *et al.*, 2007). The reason behind improvements associated with the addition of kefir might be due to the more than 50 strains of beneficial bacteria (Pogacic *et al.*, 2013). Kefir has a role in balancing gut microbes, which manipulate microorganisms and increase nutrients absorption rate (Musa *et al.*, 2009). These results were in agreement with those of Kowalski *et al.* (2009), who fed cattle with *Bacillus licheniformis* and *Bacillus subtilis*.

**Table (2) Mean of body weight (Kg) and growth rate (Kg) of lambs received different levels of kefir (± Standard Deviation)**

Treatments	Initial body weight	Weight of 1st month	Weight of 2nd month	Weight of 3rd month	Total growth rate
<b>Control (no Kefir)</b>	20.00±2.55	22.15±2.09	24.55±2.51	27.10±2.69	7.10±0.93
<b>Kefir 40 ml/head/day</b>	20.00±2.31	22.36±2.10	26.36±2.36	30.35±4.37	10.35±0.91
<b>Kefir 60 ml/head/day</b>	19.60±1.15	22.95±1.48	28.35±2.27	33.40±3.38	13.80±1.49

<b>Kefir 80 ml/head/day</b>	18.60±1.65	21.08±2.57	27.30±2.71	34.00±3.29	15.40±1.51
<b>Level of significant</b>	NS	NS	0.05	0.05	0.05

\*Mean with different subscript in the same column differ significantly at 5%, NS= not significant Feed consumption and feed conversion ratio

Feed consumption and feed conversion ratio are shown in the table (3). All treated group consumed more feed than the control group. Lambs received 60 ml or 80 ml of kefir consumed 1001.00 and 1004.33 gm respectively. Lambs received 80 ml kefir recorded the best feed conversion (4.80 kg feed/kg growth rate). These results are in correspondence with those of Jang *et al.* (2009), who fed lambs probiotics. The reason behind this improvement might be to the advance in microbial protein production and synthesis which increased provision of amino acid to the host animal (Antunovic *et al.*, 2006). These results were in agreement with those of Al-Galbi (2010), who fed Arabi lambs with 3% or 5% Iraqi probiotics consist of *Lactobacillus spp.* and *Lactobacilli spp.* and yeast.

**Table (3) Feed consumption and feed conversion ratio of lambs received different levels of kefir**

Treatments	Feed consumption			Feed conversion ratio		
	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
<b>Control (no Kefir)</b>	631.50	700.80	824.50	8.81	8.76	9.70
<b>Kefir 40 ml/head/day</b>	604.16	850.00	842.83	7.68	6.22	6.50
<b>Kefir 60 ml/head/day</b>	638.73	982.80	1001.00	5.72	6.46	6.0
<b>Kefir 80 ml/head/day</b>	571.20	986.90	1004.33	7.20	4.76	4.80

**Digestion rate of nutrients**

There were significant (P<0.05) in digestion rates of dry matter of treated groups with different levels of kefir (table, 4). All treated groups showed a digestion rate of the dry matter more than 70%, that of the control group was 67.21%. The same trend was shown by protein digestibility of different groups received kefir, lambs received 80 ml got highest protein digestibility (75.45%) followed by lambs received 60 and 40 ml (73.61 and 72.42% respectively). Control group showed the lowest protein digestibility 69.32%. However, lambs received different levels of kefir showed significant (P<0.05) fat digestibility (65.97%, 64.64%, 68.51% of lambs received 40, 60 and 80 ml kefir respectively) in comparison with control (57.37%). Fiber digestibility showed significant (P<0.05) differences due to the addition of kefir. Lambs received 60 or 80 ml kefir digested fiber more efficiently (63.51% or 66.06% respectively) than other groups.

**Table (4) Mean of nutrients digestibility rate (%) of lambs received different levels of kefir**

Treatments	Digestibility (%)			
	Dry matter	Crude Protein	Crude Fat	Crude Fiber
<b>Control (no Kefir)</b>	67.21±1.23	69.32±1.55	57.37±2.25	60.88±0.95
<b>Kefir 40 ml/head/day</b>	71.22±1.87	72.42±1.92	65.97±1.52	62.74±2.57
<b>Kefir 60 ml/head/day</b>	70.24±1.27	73.61±1.96	64.64±3.08	63.51±1.19
<b>Kefir 80 ml/head/day</b>	72.12±1.76	75.45±1.89	68.51±3.20	66.06±1.02
<b>Significant Level</b>	0.05	0.05	0.05	0.05

**\*Mean with different subscript in the same column differ significantly at 5%**

The improvement of dry matter and different nutrients digestibility were due to the role of kefir as probiotics which resulted in improvement in rumen fermentation and increase in microorganisms activities in the whole gut (Kim *et al*, 2006). As well as kefir consist many microbes contributed to increase degradation of feed fiber and change it to simple saccharides to be used as a source of carbon to its growth and increased microbial protein (Chaucheyras *et al*, 1995). Kefir has the ability to increase beneficial gut microbes which produce many organic acids such as acetic, propionic and butyric acids and elevate gut acidity. High gut acidity resulted in high digestion enzymes activity which influence efficiency of feed and its nutrients, finally reduced lag time (El-Waziry and Ibrahim, 2007). Always probiotics consist of cellulolytic bacteria used several times to improve digestion of mature ruminants (Kumar and Sirohi, 2013) as well as pre-weaning ruminants (Sun *et al*, 2010) as ruminant can't provide their needs to the required enzyme to degrade fibers. It was seen that yeast induced and activate cellulolytic bacteria in the rumen (Chiquette, 2009) as yeast consume oxygen and provide a suitable environment to anaerobic bacteria (Retta, 2016).

The present results are in agreement with those of Awassi lambs fed yeast (Haddad and Goussous, 2005) and beef goats (Whitley *et al*, 2009) fed commercial probiotics.

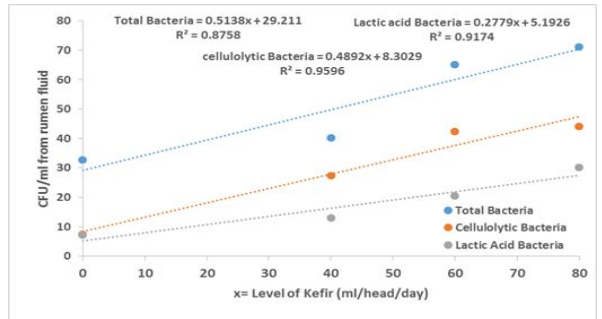
**Rumen parameters pH**

Rumen fluid pH has not changed significantly with the addition of any level of kefir (table, 5). The group received 40, 60 and 80 ml of kefir recorded pH value after 3 hours of feeding equal to 6.50, 6.73 and 6.16 respectively that of control was 7.17. While pH at 6 hours after feeding values showed nearly similar values as those at 3 hours after feeding (7.50, 6.26, 6.73, 6.40 for control, 40, 60, 80 ml of kefir respectively).

Probiotics were mainly selected to improve digestion through manipulation of rumen pH (Mohamed *et al*, 2004), improve fiber digestion (El-Waziry and Ibrahim, 2007) and microbial synthesis (Uyeno *et al*, 2015). The reason behind a static rumen pH might be due to the balance between the increase in bacteria number that fermented starch and ciliate protozoa that consumed starch (Throne *et al*, 2009; Nocek and Kautz, 2006).

**Rumen microflora**

A total number of bacteria, cellulolytic bacteria, and acetic acid bacteria differed significantly (P<0.05) after the admission of kefir milk (table, 5). A total number of bacteria increased significantly (P<0.05) with the addition of 40, 60 and 80 ml of kefir (40.29, 65.23 and 71.20 x10<sup>9</sup> CFU/ml respectively) in comparison with that of the control group (32.69x10<sup>9</sup> CFU/ml). The Same trend has been shown by cellulolytic bacteria and acetic acid bacteria. Cellulolytic bacteria number increased from 7.37x10<sup>8</sup> CFU/ml (control group) to 27.34, 42.45 and 44.11x10<sup>8</sup> CFU/ml when kefir milk was added at a level of 40, 60 and 80 ml respectively. Whereas, acetic acid bacteria recorded values of 7.21, 12.93, 20.56 and 30.00 CFU/ml for control, 40, 60 and 80 ml of kefir respectively. The increase in a number of bacteria was related significantly with level of kefir (Fig. 1). As shown in Fig. 1 each 10 ml of kefir increased number of total bacteria by 5.14x10<sup>7</sup>cfu/ml, cellulolytic bacteria by 4.90x10<sup>7</sup>cfu/ml and lactic acid bacteria by 2.78x10<sup>7</sup> CFU/ml with a confidence of 87.58%, 95.96% and 91.74% respectively. These figures indicated that kefir level contributed more than 87% of the variation of total bacteria, 95% of the variation of cellulolytic bacteria and 91% of the variation of lactic acid bacteria. These results suggest that with this type of animal and ration, kefir is the main factor to control a number of bacteria directly or indirectly by manipulation rumen ecology. Kefir as probiotics enhance bacteria growth by degrading fiber and increase energy availability (Kritas *et al*, 2006 and Tripathi *et al*, 2008).



**Fig (1) Association between different levels of kefir and number of total, cellulolytic and acetic acid bacteria**

**Table (4) Mean of rumen pH, number of totals, cellulolytic and lactic acid bacteria of lambs received different levels of kefir**

Traits	pH		Microflora (after 3 hours of feeding) CFU/ml		
	After 3 hours of feeding	After 6 hours of feeding	Total Bacteria x10 <sup>9</sup>	Cellulolytic Bacteria x10 <sup>8</sup>	Lactic Acid Bacteria x10 <sup>7</sup>
Control (no Kefir)	7.16±0.7 6	7.50±0.5 0	32.69±3.71 b	7.37±0.52 c	7.21±0.98 d
Kefir 40 ml/head/day	6.50±0.5 0	6.26±0.2 5	40.21±6.36 b	27.34±2.03 b	12.93±3.69 c
Kefir 60 ml/head/day	6.73±0.6 8	6.73±1.1 0	65.23±6.44 a	42.45±4.36 a	20.56±3.93 b
Kefir 80 ml/head/day	6.16±1.0 2	6.40±0.5 2	71.20±6.63 a	44.11±5.95 a	30.10±4.96 a
Significant Level	NS	NS	0.05	0.05	0.05

**\*Mean with different subscript in the same column differ significantly at 5%. NS= not significant Volatile fatty acids**

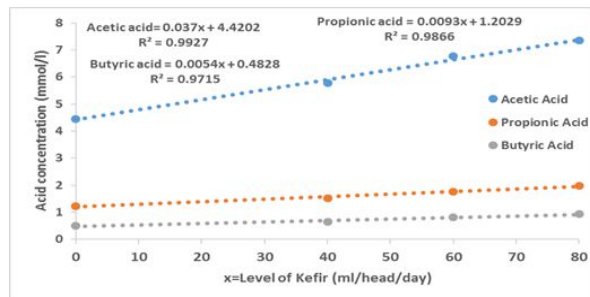
Total volatile fatty acids concentration and acetic, propionic and butyric acids percentages were shown in the table (6). Kefir addition caused significant (P<0.05) increase in total volatile fatty acids from 4.45 mmol/l recorded by control group to 7.35 mmol/l recorded by group received 80 ml kefir. Whereas, acetic acid percent decreased (P<0.05) with the increased level of kefir (71.60%, control group to 68.83%, group received 80 ml kefir). A similar trend was shown by propionic acid (7.80% and 8.80% for control and 80 ml kefir groups respectively).

**Table (6) Mean of volatile fatty acid percent (%) of lambs received different levels of kefir**

Treatments	Total fatty acid concentration (mmol/l)	Acid %		
		Acetic	Propionic	Butyric
Control (no Kefir)	4.45±0.30 c	71.60±0.22 c	18.50±0.16 b	7.80±0.11 b
Kefir 40 ml/head/day	5.77±0.87 b	70.67±0.28 b	18.60±0.16 ab	8.00±0.10 ab
Kefir 60 ml/head/day	6.77±0.61 ab	69.19±0.29 ab	18.70±0.15 ab	8.60±0.14 a
Kefir 80 ml/head/day	7.35±.65 a	68.83±0.21 a	19.00±0.17 a	8.80±0.13 a
Significant Level	0.05	0.05	0.05	0.05

**\*Mean with different subscript in the same column differ significantly at 5%**

The relationship between the level of kefir and concentration of acetic, propionic and butyric acids are shown in Fig. 2. The concentration of zero level of kefir recorded a mean of 4.42, 1.20 and 0.48 mmol/l of acetic, propionic and butyric acid respectively. An increase of 10 ml of kefir associated with an increase of 0.37, 0.09 and 0.05 mmol/l for acetic, propionic and butyric acids with a confidence of 99.27%, 98.66%, and 97.15% respectively. It is clear from these results that kefir is highly contributed to the variance of all volatile fatty acids (97-99%).



**Fig. (2) Association of kefir levels and rumen fluid volatile fatty acids**

Feeding cattle probiotics contain *propiobacterium* strain p169 bacteria resulted in a similar result as the present study result, an increase in the concentration of propionic and butyric acids with a decrease in concentration of acetic acid (Weiss *et al*, 2008). Similar results were also found when Holstein Friesian cattle fed *Bacillus licheniformis* bacteria (Qiao *et al*, 2009 and Kowalski *et al*, 2009). Increase volatile fatty acids in the rumen related to a static pH, improve rumen microflora especially the bacteria (cellulolytic bacteria), high level of feed fiber degradation and improvement in the absorption of fatty acids through rumen wall (Chaucheyras-Durand *et al*, 2012).

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