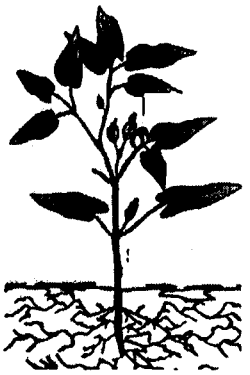

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EFFECT OF COW'S MILK TREATMENT WITH TRANSGLUTAMINASE ON THE COMPOSITION AND QUALITY OF YOGHURT WITH PARTICULAR REFERENCE TO ITS BIOLOGICAL VALUE

[18]

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ABSTRACT

The aim of this study was to evaluate the impact of cow's milk treatment with transglutaminase (TGase) enzyme on the resultant yoghurt attributes with emphasis on the biological properties.

Yoghurt was made using milk treated previously with TGase at levels of nil (the control), 250 and 500 units/l yoghurt milk at 40°C for 2 h. Then, the enzyme was inactivated by heating, inoculated with 2% of freshly activated yoghurt starter culture (YSC), and incubated until complete coagulation.

The obtained results indicate that the TGase treatment of yoghurt milk led to significant increments in the contents of dry matter (DM), protein/DM, lactose/DM, ash/DM, Ca, P, K, Mg, Cu and Zn, the amino acid (AA) threonine and all non essential (E) AA, except of histidine, true digestibility %, biological value % and net protein utilization %.On the contrary, the fat/DM content, Fe, Mn, vitamin B_2, glutamic acid and EAA lysine, methionine, cystine, leucine, isoleucine, phenylalanine, tyrosine and valine decreased, while those of Na, vitamin B_1 and the growth rate of rats fed on TGase treated yoghurts were not changed. The growth of both strains of YSC slowed significantly down as TGase level was raised. TGase treatment of yoghurt milk led to obtain a yoghurt body with higher values of consistency coefficient and yield stress *versus* the control. The blood picture of rats fed on TGase treated yoghurts did not exhibit any significant differences either in the hemoglobin value or hematocrit %. While the red blood cell and the white blood cell counts were significantly higher in the blood of rats fed on TGase treated sample. The change rates in most attributes became more pronounced as TGase dose was progressed. Rats feeding on TGase treated yoghurt was associated with significant increment in the total as well as globulin protein and in levels of Ca, K, Mg, Cu, Fe and Zn in their blood plasma. On the contrary, the ratio between them appeared proportional decrement related to the enzyme dose. The feeding on yoghurt led to reduce in the *E. coli* count of rats' feces and to obvious predominance of the yoghurt starter strains.

From the foregoing results it can be conclude that, TGase treatment of yoghurt milk gave a product of better consistency and improved the protein and mineral bioavailability and blood and blood plasma pictures of experimental animals.

INTRODUCTION

Set yoghurt made from cow's milk is usually characterized with a pronounced weak body. In order to overcome this problem, milk solids are

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raised either by evaporation, membrane filtration or addition of skim milk powder, or protein concentrates **(Abou-Donia, 1984; Haggag & Fayed, 1988; Fayed *et al* 1996; Tamime & Robinson, 1999; Remeuf *et al* 2003 and Youssef *et al* 2007).**

Recently, some reports recommended the use of enzymatic cross linking of milk proteins, in order to improver the texture of yoghurt **(Kuraishi *et al* 1996 & 2001; El-Kenany, 2003; Abou El-Nour *et al* 2004; Husein *et al* 2006 and Ozer *et al* 2007).** The enzyme used for this purpose is transglutaminase (TGase) which catalyses the formation amide group between the γ-carboxylic group of glutamic acid and ε- amino group of lysine. An acyl-transfer reaction in which the γ-carboxamide groups of peptide bound glutaminyl residues are the acyl donors. As a result of cross-linking of peptide-bound glutamine and lysine residues, ε-(γ-glutaminyl) lysine isopeptide bonds and high molecular weight polymers are formed **(Zhu *et al* 1995 and Sharma *et al* 2001).** Yoghurt made from TGase treated milk had a greater capacity for holding water, increased gel strength and whey syneresis prevented **(Ishii *et al* 1994; Husein *et al* 2006 and Ozer *et al* 2007).**

Nevertheless, some reservation confirmed that the ε-(γ-glutaminyl) lysine moiety is partically resistant to digestion by mammalian gastrointestinal enzymes **(Lorenzen, 2002).** For that in view, the aim of this study was to evaluate the effect of milk treatment with TGase on the biological value of milk proteins as well as the composition and rheological properties of yoghurt.

MATERIAL AND METHODS

Materials

Fresh cow's milk (3.50% fat and 3.27% protein) was obtained from the herd of Higher Institute of Agric. Co-operation, Shoubra El-Kheima. A Ca – independent microbial transglutaminase from *Streptoverticillium mobaraense* (ACTIVA® MP, with activity of 100 units/g powder) was obtained from Ajinomoto Europe Sales GmbH, Hamburg, Germany. The declared chemical composition of ACTIVA® MP preparation was as follow: 1.1% moisture, 0.8% protein (TN X 6.25), <0.1% fat, 0.1% ash, 98.0% available carbohydrate, and <0.5% Dietary fiber. Lyophilized mixed yoghurt starter culture (YSC) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from Chr. Hansen's laboratories, Copenhagen, Denmark.

Experimental procedures

Yoghurt milk was heated to 85°C for 5 min and then cooled to 40°C. TGase was added at a level of nil (the control), 250 or 500 units/L yoghurt milk. After 2 h. at this temperature degree, the reaction was stopped by heating TGase-containing milks again at 90°C for 5 min in order to inactivate the enzyme as recommended by **Ozer *et al* (2007)** and then cooled to 42°C. All milks were converted into yoghurt according to the protocol proposed by **Tamime & Robinson (1999)** with adopting the manufacture conditions enacted by **EOSQ (2005),** where milks were inoculated with 2% of freshly activated YSC, filled into 1 kg polystyrene containers, covered, and incubated at the same temperature degree (42°C) until complete coagulation (~ 3 h.). Thereafter, the containers were transferred to the refrigerator (5±1°C), where they were kept to the next day for analyses. While, the lots subjected for the biological assay were spray dried as described by **Hussein *et al* (2008)** using A/S Niro Atomizer, Copenhagen, Denmark, NO. 1295. Three replicates were made from each treatment.

Methods of analysis

Dry matter (DM), fat, nitrogen and ash contents as well as titratable acidity (TA) were determined according to **AOAC (2007).** Lactose content was calculated by the difference. The acetaldehyde (AC) content was estimated as described by **Lees & Jago (1969).** The pH value was measured using a pH meter (HANNA Instruments, USA). Amino acids other than tryptophan were determined as in **Moore *et al* (1958)** using a Beckman High Performance Amino Acid Analyzer system 7300 with a column Na-E/F/D 25 cm. and Data system 7000.

Rheological properties of yoghurt were measured at 10°C using a rotary viscometer (RHEOTEST, type RV and Pruefgeraetewerk Medingn, Dresden) as described by **Toledo (1980).** Consistency coefficient was calculated from the descending flow curve using the following equation:

$$Log\delta = log\ \kappa + n\ log\ \gamma$$

Where

δ = Shearing stress, γ = shear rate,
n = Flow behaviour index and κ = consistency coefficient.

Whilst, the yield stress was calculated by fitting the shear stress-shear rate data to the Casson equation (**Bourne, 1982**).

$$\sqrt{\delta} = \sqrt{\delta o} + \eta a \sqrt{\gamma}$$

Where

δ = Shearing stress, δo = yield stress, ηa = apparent viscosity, γ = shear rate.

Calcium (Ca), phosphorus (P), potassium (K), sodium (Na) and magnesium (Mg), zinc (Zn), iron (Fe), cupper (Cu) and manganese (Mn) contents were determined using an Inductive Coupled Plasma (ICP)-plasma technique, Perkin Elmer-Optima 2000 DV as described in **AOAC (2007).**

The counts of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were determined by enumeration on M17 and MRS agar media, respectively as described by **Gueimonde *et al* (2003).** Coliform was counted using violet red bile agar (VRBA) at pH 7.0-7.2, 37°C for 2 days as described by **Hitchins *et al* (1992).**

Thiamin (B_1) and Riboflavin (B_2) were measured as described by **Bognar (1992)**, using Beckman HPLC consisting of pump model 126, injector and data handling system. Perkin-Elmer fluorescence detector LC240 and C18 column 25 cm x 4.6 mm were used.

The biological value of yoghurt was assessed in a feeding experiment using three groups of Albino male rats (5 animals for each) of 70-75g **(Schiller, 1960 and Horszczaruk & Bock, 1963).** The first group of animals received a basic diet, while the 2nd and 3rd group received diet containing yoghurt and TGase-yoghurt, respectively. The composition of diet for the three groups is given in **Table (1).**

Table 1. Composition of feeding iso-caloric diet providing 150 mg nitrogen, 40 kcal. and10 g dry matter (DM)/rat/day based on cow's milk yoghurt treated with different transglutaminase (TGase) doses.

Constituent (g)	Unit of TGase / kg yoghurt milk		
	Nil (control)	250	500
Dried yoghurt (95% DM)	3.484	3.462	3.444
Starch	5.273	5.310	5.330
Sucrose	0.568	0.568	0.568
Cellulose	0.328	0.328	0.328
Minerals mixture *	0.447	0.432	0.430
Vitamins mixture *	0.100	0.100	0.100
Total diet/ rat/ day	10.200	10.200	10.200

* Mixtures were composed of as in **Eggum (1973).**

The feeding experiment was carried out for 9 days. The animals were weighed at the beginning and end of the experiment. Also, urine and feces were collected for N analysis **(Waynforth & Flecknell, 1992).** At the end of the experiment animals were anaesthetized and blood samples were collected. Total protein and albumin of blood plasma were measured photometrically using the biuret and bromocresol green methods in order **(Johnson *et al* 1999),** and globulin were calculated by the difference between them. Complete blood picture was carried out according to the User's Manual of **RDS (1995)** using Micro-Cobas automatic blood cell counter.

The parameters of the protein quality, namely true digestibility (TD) biological value (BV) and net protein utilization (NPU) were determined according to **Eggum (1973).**

The obtained data were statistically analyzed according to statistical analyses system user's guide **(SPSS, 1998).**

RESULTS AND DISCUSSION

Gross composition

The obtained results given in **Table (2)** indicate that the TGase treatment of yoghurt milk significantly increased in the dry matter (DM), protein/DM, lactose/DM and ash/DM contents and decreased the fat/DM content of the resultant yoghurt. This can be attributed to the increase in DM brought by the added TGase preparation. Theoretically, the DM of yoghurt treated with 250 unit/L would increase by 0.25% while that treated with 500 unit/L would increase by 0.5%. The increased DM was making carbohydrate which may explain the increase in the lactose content. Also, the retardation in lactose fermentation in TGase-treated yoghurt evident from the low acid development may add to the increased lactose content in yoghurt. However, this increment came only in the expanse of the fat/DM content other than other components levels because the added enzyme powder is considered as supplement for proteins and minerals. The overall trends of these results agree with those reported by **Neve *et al* (2001); Lorenzen *et al* (2002); Abou El-Nour *et al* (2004) and Husein *et al* (2006).**

Bacterial population

Concerning the yoghurt milk treatment with TGase in relation to the bacterial count of YSC

enumerated in one-day age yoghurt, data show that the growth of both strains slowed significantly down as TGase level was raised (**Table 2**). Similar observation were reported by **Neve *et al* (2001)**, who declared that the enzymatic cross-linking step led to a minor imbalance of the association growth (i.e. the proto symbiosis) in the strains of the YSC. The reduction in cell counts correlated well with the delay in the pH in the enzyme treated samples. Similar findings were recorded also by **Lauber *et al* (2000); Lorenzen *et al* (2002); Husein *et al* (2006) and Ozer *et al* (2007)**, who reported that cross- linking of milk protein by TGase slowed down the growth of yoghurt starter bacteria.

Table 2. Chemical, rheological and bacterial properties of enzymatic treated cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Property	Unit of TGase / kg yoghurt milk		
	Nil (control)	250	500
Dry matter (DM)%	12.85^{b}	13.03^{a}	13.10^{a}
Fat/DM %	30.26^{a}	29.52^{b}	29.39^{b}
Protein/ DM %	28.92^{b}	29.10^{ab}	29.25^{a}
Lactose*/ DM%	28.73^{b}	29.81^{ab}	30.00^{a}
Ash/ DM%	6.25^{b}	6.35^{a}	6.40^{a}
Acidity** %	0.75^{a}	0.68^{ab}	0.65^{b}
pH value	4.50^{b}	4.70^{a}	4.75^{a}
AC (µmol/100 g)	308^{a}	245^{b}	163^{c}
CC (dyne. sec./ cm^2)	14.80^{c}	15.64^{b}	16.25^{a}
YS (dyne/cm^2)	40.94^{c}	53.21^{b}	62.48^{a}
Str. (cfu x.10^7)	19.50^{a}	18.94^{b}	18.62^{c}
Lb. (cfu x.10^6)	19.06^{a}	18.00^{b}	16.98^{c}

The means with the same letter within the same row did not significantly differ (P>0.05).
* Calculated by the difference.
** Determined as lactic acid.
AC: Acetaldehyde CC: Consistency coefficient
YS: Yield stress
Str.: *Streptococcus thermophilus*
Lb.: *Lactobacillus delbrueckii* subsp. *bulgaricus*.
cfu: colony forming unit.

Biochemical properties

The decrease in the bacterial population of YSC was clearly reflected on the biochemical properties of the resultant yoghurt through a late development of acidity and acetaldehyde, and a corresponding delayed drop of pH value due to TGase treatment. These changes became more pronounced as the enzyme dose was increased (**Table, 2**). The trends of these results agree with those found by **Neve *et al* (2001); Lorenzen *et al* (2002); Abou El-Nour *et al* (2004); Husein *et al* (2006) and Ozer *et al* (2007)**, who reported that the development of TA and AC yoghurt were slowed down by TGase.

Rheological properties

As seen in **Table (2)**, TGase treatment of yoghurt milk led to texture of high consistency coefficient (CC) and yield stress (YS) values *versus* the control. Furthermore, there are a positive relationship between the enzyme dose and the two rheological indices. **Lorenzen & Neve (2002) and Kirmaci *et al* (2004)** explained that the enzyme-induced increase in viscosity of yoghurt might be due to a reduction of pore sizes of the protein network. Similar findings were reported with respect to yoghurt viscosity by **Abou El-Nour *et al* (2004); Husein *et al* (2006) and Ozer *et al* (2007).**

Micronutrients

Regarding the minerals and trace elements content of yoghurt, data illustrated in **Table (3)** appear that, TGase adding to yoghurt milk at the experimental levels yielded in significant increments in the levels of Ca, P, K, Mg, Cu and Zn which may be attributed to the carry over from the added TGase powder. On the contrary, the levels of Fe and Mn were decreased, while that of Na was not changed. **Tsai *et al* (1996) and Motoki & Seguro (1998)** explained that Cu and Zn bind the thiol group of the single cystein residue of enzyme molecule.

Although the level of vitamin B_1 was not influenced, a significant reduction was found in the level of vitamin B_2 being correlated to the yoghurt treatment with TGase.

The obtained levels of micronutrients in the control yoghurt are in reported rang of these constituents to yoghurt **(Rasic & Kurmann, 1978; Tamime & Robinson, 1999 and Youssef *et al* 2007).**

Table 3. Micronutrients of enzymatic treated cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Component	Unit of TGase / kg yoghurt milk		
	Nil (control)	250	500
Minerals(g/kg)			
Ca	1.444^{b}	1.512^{ab}	1.657^{a}
P	1.117^{b}	1.210^{ab}	1.284^{a}
K	1.457^{c}	1.845^{b}	2.188^{a}
Na	0.438^{a}	0.440^{a}	0.446^{a}
Mg	0.125^{b}	0.132^{ab}	0.140^{a}
Trace elements (mg /kg)			
Cu	0.363^{b}	0.389^{ab}	0.403^{a}
Fe	0.340^{a}	0.325^{ab}	0.303^{b}
Zn	5.082^{b}	5.351^{ab}	5.780^{a}
Mn	0.106^{a}	0.100^{ab}	0.095^{b}
Vitamin (mg/kg)			
B_1	0.496^{a}	0.485^{a}	0.480^{a}
B_2	1.539^{a}	1.390^{b}	1.284^{c}

-The means with the same letter within the same row did not significantly differ (P>0.05).

Amino acids composition

The results present in **Table (4)** demonstrate that there are significant reductions in their summations due to TGase treatment, where both of lysine, methionine, cystine, leucine, isoleucine, phenylalanine, tyrosine and valine percentages decreased and that of threonine increased. The change rate became more pronounced at high TGase dose. This reduction in the EAA of TGase-treated yoghurt protein may be due to the interaction between some AA occurred by TGase and also due to the relatively low content of EAA consisting of the enzyme used (113 EAA out of 331 totals (T) AA **(Kanaji *et al* 1993)**. Therefore, the EAA/TAA ratio was consequently significantly lowered **(Table 4).**

On the other hand, TGase itself is relatively rich in arginine, serine, & glutamine, aspartic acid & asparagen, glucine, alanine and praline valuing together 210 out of 331 TAA **(Kanaji *et al* 1993).** Thereby all non EAA, except of histidine, increased in TGase-treated yoghurt protein **(Table 4)**. However, glutamic acid content of yoghurt protein decreased significantly by TGase treatment. **Kanaji *et al* (1993)** found that TGase molecule contains 23 glutamic acid residues out of 331 AA. The general AA composition of the control sample agrees with that reported by **Rasic & Kurmann (1978) and Youssef (1993).**

Table 4. Amino acid composition (g/100 protein) of cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Amino acid	Unit of TGase / kg yoghurt milk		
(AA)	Nil (control)	250	500
Essential (E)			
Lysine	8.53^{a}	7.94^{b}	7.67^{c}
Methionine	2.86^{a}	2.72^{ab}	2.61^{c}
Cystine	0.92^{a}	0.88^{ab}	0.82^{b}
Threonine	4.22^{b}	4.36^{ab}	4.54^{a}
Isoleucine	4.67^{a}	4.41^{ab}	4.28^{b}
Leucine	9.25^{a}	8.88^{b}	8.36^{c}
Phenylalanine	4.83^{a}	4.74^{ab}	4.58^{b}
Tyrosine	5.82^{a}	5.41^{ab}	5.05^{b}
Valine	6.11^{a}	6.02^{ab}	5.95^{b}
Tryptophan	ND	ND	ND
Sum of EAA	47.21^{a}	45.36^{b}	43.86^{c}
Non-essential			
Histidine	2.69^{a}	2.60^{ab}	2.50^{b}
Arginine	3.91^{c}	4.25^{b}	4.62^{a}
Serine	4.74^{c}	5.31^{b}	5.81^{a}
Glutamic acid	21.56^{a}	20.82^{b}	20.04^{c}
Asparatic acid	6.50^{c}	7.14^{b}	7.97^{a}
Glycine	1.96^{c}	2.18^{b}	2.39^{a}
Alanine	2.67^{c}	2.80^{b}	2.99^{a}
Proline	8.27^{c}	8.92^{b}	9.44^{a}
Total (T) AA	99.51^{c}	99.38^{a}	99.62^{a}
EAA/TAA ratio	47.44^{a}	45.64^{b}	44.03^{c}

The means with the same letter within the same row did not significantly differ (P>0.05).

Nutritional protein quality

Table (5) indicates a gradual improvement in the protein quality associated with the dose of TGase used in yoghurt-milk treatment. This was apparent from the increase in all parameters de-

termined namely true digestibility %, biological value % and net protein utilization %. Nevertheless, the growth rate was not affected by the TGase treatment. Data of the control sample are in complete agreement with those reported by **Youssef *et al* (2007).**

Table 5. Nutritional protein quality and growth rate of rats fed on cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Property	Unit of TGase / kg yoghurt milk		
	Nil (control)	250	500
True digestibility %	93.68^{c}	96.20^{b}	99.07^{a}
Biological value %	85.74^{c}	89.00^{b}	92.98^{a}
Net protein utilization %	80.32^{c}	85.62^{b}	92.12^{a}
Growth rate (g/day)	2.10^{a}	2.11^{a}	2.11^{a}

The means with the same letter within the same row did not significantly differ (P>0.05).

Blood picture

The blood picture of rats fed on TGase treated yoghurts did not exhibit any significant differences either in the hemoglobin (HGB) value or hematocrit (HCT) % from those fed on the enzyme untreated one **(Table 6)**. While the red blood cell (RBC) and the white blood cell (WBC) counts were significantly higher in the blood of rats fed on TGase treated sample and as TGase dose raised. This could be explained by the high absorption of most minerals and trace elements by feeding on TGase treated yoghurt **(Table 7)**. These elements would promote formation of WBC. **Harris (1997) and Turnlund (1999)** declared that two copper-containing enzymes, ceruloplasmin (ferroxidase I) and ferroxidase II have the capacity to oxidize ferrous iron (Fe2+) to ferric iron (Fe3+), the form of iron that can be loaded onto the protein transferrin for transport to the site of red blood cell formation. Although the ferroxidase activity of these two cuproenzymes has not yet been proven to be physiologically significant, the fact that iron mobilization from storage sites is impaired in copper deficiency supports their role in iron metabolism. Likewise, Zinc is required for the development and activation of T-lymphocytes, a kind of WBC component. All blood picture parameters of rats fed on the control samples are in coincidence with those found by **Youssef *et al* (2007).**

Table 6. Blood picture of rats fed on cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Property	Unit of TGase / kg yoghurt milk		
	Nil (control)	250	500
White blood cell (10^3/mm^3)	5.82^{c}	7.00^{b}	8.25^{a}
Red blood cell (10^6/mm^3)	6.58^{c}	6.67^{b}	6.84^{a}
Hemoglobin (g/dl)	12.55^{a}	12.42^{a}	12.38^{a}
Hematocrit %	36.84^{a}	37.15^{a}	37.40^{a}

The means with the same letter within the same row did not significantly differ (P>0.05).

Blood plasma profile

Table (7) show that, feeding rats on TGase treated yoghurt was associated with significant increment in the total as well as globulin protein depending positively on enzyme dose. However, the albumin level was not influenced. On the contrary, the ratio between them appeared proportional decrement related to the enzyme dose.

Regarding to the mineral composition of blood plasma data given in **Table (7)** indicates that the feeding rats on TGase treated yoghurt improved the intestinal permeability, where there are significant increment increase in levels of Ca, K, Mg, Cu, Fe and Zn. **Murphree (2003)** reviewed that glutamine improved intestinal permeability. Where, glutamine is converted to Glutamic acid in the brain. Glutamic acid increases neuronal activity, detoxifies ammonia (an abundant waste product in the body) from cells, and like glucose, is used to feed the brain. L-glutamine plays an important role in intestinal maintenance and repair. Glutamine is the major energy source of the intestines. It is one of the most important nutrients for the cells that line the colon. It is worthy to mention that TGase molecule consists of 10 glutamine residues out of 331 AA **(Kanaji *et al* 1993)**. Moreover, some AA play also a positive role in this respect where, **Wasserman *et al* (1956)** confirmed earlier that L-arginine was the most potent in promoting mineral absorption, approximately doubling the Ca^{45} found in the femurs. L-leucine,

and L-aspartic acid also produced notable increases. Noteworthy, as previously mentioned TGase treated yoghurts contained those AA at levels higher than those of untreated samples **(Table, 4)**. The relatively increased lactose level possessed of TGase treated yoghurt *versus* the untreated one **(Table, 2)** may imparts a further reason to explain this phenomenon. **Wasserman *et al* (1956)** reported that lactose produced a greater response than L-arginine in promoting mineral absorption. Recently, **Fayed *et al* (2006)** reviewed that the increased intestinal acidity was aiding absorption of minerals, especially calcium. While, the level of P was not changed by TGase treatment.

Table 7. Blood plasma profile of rats fed on cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Property	Unit of TGase / kg yoghurt milk		
	Nil (control)	250	500
Total protein (g/dl)	5.23^{b}	5.35^{ab}	5.45^{a}
Albumin (A) (g/dl)	3.38^{a}	3.38^{a}	3.39^{a}
Globulin (G) (g/dl)	1.85^{b}	1.94^{ab}	2.06^{a}
A/G ratio	1.83^{a}	1.74^{ab}	1.65^{b}
Mineral (ppm)			
Ca	53.82^{c}	74.41^{b}	92.80^{a}
P	176.85^{a}	176.04^{a}	175.20^{b}
K	120.85^{c}	140.73^{b}	160.90^{a}
Mg	11.628^{c}	13.070^{b}	15.950^{a}
Cu	0.695^{c}	0.782^{b}	0.900^{a}
Fe	3.092^{c}	4.821^{b}	6.420^{a}
Zn	1.125^{c}	1.414^{b}	1.640^{a}

The means with the same letter within the same row did not significantly differ (P>0.05).

Intestinal flora

As seen in **Table (8)**, the feeding on yoghurt led to reduction in the *E. coli* count of rats' feces and to obvious predominance of the yoghurt starter strains. Similar results were reported by **Hansen (1985) and Fayed *et al* (2006).** Nevertheless, there was a pronounced decrease in this predominant population due to the feeding on TGase treated yoghurt, and hence the chance to *E. coli* to multiply was restored. The relatively harmful effect of TGase on the growth YSC was previously found during yoghurt production **(Table, 2)** and discussed.

Table 8. Some feces flora (cfu×10^3 /g) of rats fed on cow's milk yoghurt as a function of transglutaminase (TGase) dose.

Strain	Unit of TGase / kg yoghurt milk					
	Nil (control)		250		500	
	Before	After	Before	After	Before	After
Str.	ND	25.0^{a}	ND	10.80^{b}	ND	1.0^{c}
Lb.	ND	33.5^{a}	ND	15.43^{b}	ND	1.8^{c}
E.coli	120.5^{b}	27.5^{a}	117.4^{a}	100.6^{b}	114.5^{b}	170.0^{a}

The means with the same letter within the same row did not significantly differ (P>0.05).
Str.: *Streptococcus thermophilus*
Lb.: *Lactobacillus delbrueckii* subsp. *bulgaricus*
Before: prior the experimental feeding.
After: at the end of the experimental feeding.
ND: not detected. cfu: colony forming unit.

From the foregoing results it can be conclude that, TGase treatment of yoghurt milk gave a product of better consistency and improved the protein and mineral bioavailability and blood and blood plasma pictures of experimental animals.

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حوليات العلوم الزراعية
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تأثير معاملة اللبن البقرى بأنزيم الترانس-جلوتاميناز على تركيب وجودة اليوجهورت وبخاصة قيمته الحيوية

[١٨]

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استهدفت الدراسة تقييم تأثير معاملة اللبن البقري بأنزيم الترانس جلوتاميناز على خواص اليوجهورت الناتج وخاصة الخواص الحيوية

حيث تم تصنيع اليوجهورت من لبن معامل بالأنزيم بمستويات صفر (الكنترول)، ٢٥٠، ٥٠٠ وحدة/ لتر لبن على درجة حرارة ٤٠°م لمدة ساعتين. ثم تم وقف نشاط الأنزيم بالتسخين حيث تم تلقيح اللبن بنسبة ٢% من بادئ اليوجهورت والتحضين حتى تمام التجبن.

وقد دلت النتائج المتحصل عليها على إنه بمعاملة لبن اليوجهورت بالأنزيم زادت معنوياً نسب الجوامد الكلية، البروتين/ المادة الجافة، اللاكتوز/ المادة الجافة، الرماد/ المادة الجافة، الكالسيوم، الفوسفور، البوتاسيوم، الماغنسيوم، النحاس، الزنك، الحمض الأميني الثيريونين وكل الأحماض الأمينية غير الأساسية ما عدا الهيستيدين. كما زاد معدل الهضم والقيمة الحيوية وصافي الاستفادة بالبتروجين البروتين. وعلى العكس انخفضت نسبة الدهن/ المادة الجافة، الحديد، المنجنيز، فيتامين ب٢، حمض الجلوتاميك والأحماض الأمينية الأساسية ليسين، ميثيونين، سستئين، ليوسين، ايزوليوسين، فينيل آلانين، تيروزين والفالين. في حين لم تتأثر نسب الصوديوم، فيتامين ب١ ، ومعدل نمو الفئران بمعاملة لبن اليوجهورت بالأنزيم.

بينما ظهر بطء في نمو بكتيريا بادئ اليوجهورت كلما زادت جرعة الأنزيم المستخدمة. وكذلك فإن المعاملة بالأنزيم أدت إلى زيادة معامل القوام وجهد القص الابتدائي بالمقارنة بالكنترول.ولم تظهر صورة دم الفئران أى اختلاف نتيجة التغذية على اليوجهورت المعامل بالأنزيم وذلك في قيم الهيموجلوبين أو نسبة الهيماتوكريت (النسبة الحجمية للكرات الدموية الحمراء بالدم) بينما زاد عدد كرات الدم الحمراء والبيضاء نتيجة لذلك. وبفحص بلازما الدم وجد أن هناك زيادة في مستويات البروتين الكلي والجلوبيولين وفي نسب الكالسيوم، البوتاسيوم، الماغنسيوم، النحاس، الحديد والزنك مصاحبة للتغذية على اليوجهورت المعامل بالأنزيم. وعلى العكس إنخفضت النسبة بين الألبيومين/ الجلوبيولين بارتفاع جرعة الإنزيم المستخدمة في اليوجهورت.ولقد ظهر أن التغذية على اليوجهورت بصفة عامة أدى إلى خفض إعداد بكتيريا القولون وزيادة أعداد سلالات بكتيريا بادئ اليوجهورت في براز الفئران.

ومما سبق يمكن الاستنتاج بأن معاملة لبن اليوجهورت بأنزيم الترانس جلوتاميناز أضفى على المنتج قوام أفضل كما أنه حسن من معدل إمتصاص العناصر والاستفادة من البروتين وكذلك صورة الدم وبلازما الدم فى الحيوانات المختبرة.

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