

DETERMINATION OF UREA IN COW'S MILK SOLD IN DAMIETTA GOVERNORATE

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SUMMARY

Fifty random samples of fresh cow's raw milk present as bulk tank milk were collected from different street vendors, groceries and dairy shops at Damietta Governorate. The samples were submitted for analysis colorimetrically by using spectrophotometer to determine the levels of added urea concentration. The analysis was performed before and after heat-treatment by boiling method.

The obtained results revealed that mean value of urea concentrations in fresh cow's raw milk samples was found to be 18.047 ± 0.203 mg/dl, while the average value of the same samples after boiling was 17.44 ± 0.198 mg/dl.

From obtained results, cow's raw milk present as bulk tank milk, sold in Damietta Governorate, considers healthy and safe for human consumption referring the normal level of milk urea nitrogen (10–16 mg/dl) and values in excess of 20 mg/dl are judge abnormal.

The obtained results and public health implications and suggestive measures were discussed.

INTRODUCTION

Liquid milk is a common health drink consumed by people of all age groups. A large population in our country depends on milk from local suppliers. Milk is a product of biological evolution. It is a biochemical complex which appears to be the only material to function solely as a source of food. The complements of proteins in milk are ideal in quality and balance to satisfy human amino acid requirements. Confirmation of this nutritive image is the widespread use of milk and milk products as a part of the daily diet of peoples in the highly developed countries. As a consequence, such societies enjoy almost complete freedom from nutritional disease among infants, children, young and adults. In contrast, the poorly or underdeveloped areas of the world have a primitive or nonexistent milk supply and have numerous inhabitants suffering from nutrient deficiency, especially infants and children (**Kon, 1959; Cashman, 2002 and Hoppe et al., 2006**).

Milk considered as an excellent medium for microbial growth. This demands high standards of hygiene in its production and distribution. Due to the increasing demand, adulteration of milk by urea as a nitrogen compound has been very common in developing countries. Pointed out that urea was commonly added to increase both the shelf life and also milk solid not fat (SNF) value or its total nitrogen content. Although, urea is a normal constituent of milk and comprises part of the non-protein nitrogen (NPN) normally found in milk (**Ferguson, 2000**). When urea was added, the milk looked thick and concentrated giving a feeling of rich milk while, actually it was low in

fat and SNF and was poisonous (hazard toxic) due to the presence of excess urea (**FAD, 2005** and **Renny et al., 2005**).

Urea is a small organic water soluble molecule composed of carbon, nitrogen, oxygen, and hydrogen (weight 60.06 g/mol) of chemical formula $\text{CO}(\text{NH}_2)_2$ and is also known as carbamide. Other names include carbamide resin, isourea, carbonyl diamide, and carbonyldiamine (**Ferguson, 2000** and **Jenkins et al., 2002**).

A recent Indian Council of Medical research (ICMR) report has suggested that urea adulterated item has a cancerous effect on the human system and can lead to gradual impairment of the body (**Dean, 1985** and **Fox, 1992**).

Therefore, it is very important from the public health of view to evaluate the detection of any additions of urea to fresh cow's raw milk for purpose of increasing its both the shelf life and total nitrogen content and at the same time its detection after boiling as a common heat treatment at home, at Damietta Governorate.

MATERIAL AND METHODS

Sampling:

Fifty random samples of fresh cow's raw milk present as bulk tank milk were collected from different street vendors, groceries and dairy shops at Damietta Governorate. The samples (each, 500 ml) were labeled and kept in insulated ice box ($4\pm 1^\circ\text{C}$) then transferred to the laboratory for analysis.

Detection of heat-treated milk:

Each sample of raw milk was subjected to Guaiac test recommended by (**Schonberg, 1956**) to prove that the milk samples were raw.

Determination of urea before and after heat-treatment by boiling method:

These are based on the classical spectrophotometric method recommended by (**Bector et al., 1998**) that involves precipitation of proteins and lipids with trichloroacetic acid and treatment of the filtrate with dimethylamino benzaldehyde (DMAB) reagent. The blank was set and the samples were measured colorimetrically using spectrophotometer [**Jenway 6105-England**] at wavelength 425 nm. The concentration of urea was estimated from a constructed standard calibration curve and the obtained results were recorded.

Statistical analysis:

Maximum; minimum; mean and standard errors were calculated according (**SPSS, 1993**).

RESULTS AND DISCUSSION

It is noticeable from the results outlined in **table (1)** that, mean urea concentrations in 50 (100%) examined cow's raw milk present as bulk tank milk samples sold in Damietta Governorate was 18.047 ± 0.203 mg/dl with minimum concentration of 15.8 mg/dl and maximum concentration of 20.56 mg/dl. Although, these results lie around the normal level of milk urea nitrogen (MUN), measured as nitrogen in urea, (10–16 mg/dl) that mentioned by (**Jonker et al., 1998; Ferguson, 2000; Kohn, 2000; Payne, 2001; Jonker et al., 2002**) and also, according to Ontario Dairy Herd Improvement [DHI] (**Calberry, 2003**), and values in excess of 20 mg/dl are considered abnormal.

Table (1): Statistical analytical results of urea concentrations in examined cow's raw milk present as bulk tank milk samples (n=50).

Addition	Cow's raw milk (bulk tank milk) before heat-treatment by boiling method				
	Positive samples		Concentrations (mg/dl)		
	No.	%	Minimum	Maximum	Mean \pm S.E.
Urea	50.00	100.00	15.8	20.56	18.047 \pm 0.203

As far as we knew, little available data dealing with the detection of urea added to raw milk. Higher result 53.36 mg/dl was found by (Bector et al., 1998). On contrary, none of the milk samples had any added urea detected in them by (Sethi, 2005).

Table (2): Statistical analytical results of urea concentrations in examined cow's raw milk present as bulk tank milk samples after heat-treatment by boiling method (n=50).

Addition	Cow's raw milk (bulk tank milk) after heat-treatment by boiling method				
	Positive samples		Concentrations (mg/dl)		
	No.	%	Minimum	Maximum	Mean \pm S.E.
Urea	50.00	100.00	15.10	20.25	17.44 \pm 0.198

To our knowledge, there is no information available about the effect of heat treatment on urea added to milk has not been reported previously. **Table 2**, showed that mean urea concentration after heat-treatment by boiling method in 50 (100%) examined cow's raw milk present as bulk tank milk samples was 17.44 \pm 0.198 mg/dl with minimum concentration of 15.10 mg/dl and maximum concentration of 20.25 mg/dl.

It was evident that there was no significant difference in urea concentrations before and after heat treatment of milk this could be attributed to stability of urea at the boiling milk temperature (melting point of urea is 132-135 °C) (Lacks, 1979).

Normal level of milk urea nitrogen (MUN), measured as nitrogen in urea is between 10–16 mg/dl (1deciliter = 100 ml) (Jonker et al., 1998; Ferguson, 2000; Kohn, 2000; Payne, 2001; Jonker et al., 2002) and also, according to Ontario Dairy Herd Improvement (DHI) (Calberry, 2003), and values in excess of 20 mg/dl are considered abnormal.

In general, ranging of normal level of milk urea nitrogen (MUN) is know to vary according to many factors including, yielding cows have been shown to have slightly

higher levels of MUN, possibly because they are more likely to metabolize body proteins due to an energy deficit (**Carlsson et al., 1995; Broderick & Clayton, 1997** and **Godden et al., 2001**). MUN levels have been observed to change during a lactation period (**Carlsson et al., 1995** and **Godden et al., 2001**) and over subsequent lactations (**Broderick & Clayton, 1997** and **Godden et al., 2001**). Values of MUN are generally higher for grazing animals than for those fed a balanced mixed ration (**Carlsson et al., 1995**) usually due to a higher proportion of crude protein in the forage. Reports are inconsistent with respect to the effects of age and breed on MUN (**Carlsson et al., 1995**) as are reports on the effects of microbial infection in the udder (**DePeters & Ferguson, 1992**) and **Gustafsson & Palmquist, 1993**). In addition, temporal variations in milk urea are more pronounced and consistent than those due to age, breed, parity, amount of urine excreted, amount of water intake, dry matter intake, sampling methods, and days in milk (**Gustafsson & Palmquist, 1993; Carlsson & Bergström, 1994; Carlsson et al., 1995; Broderick & Clayton, 1997** and **Rodriguez et al., 1997**). Variation in MUN over the course of a single milking has been shown to be primarily due to displacement of water and water soluble components by the higher fat content in later fractions (**Carlsson & Bergström, 1994**). Seasonal changes in MUN have also been recorded, especially when cows are grazed for part of the year (**Wolfschoon-Pombo & Klostermeyer, 1982**). Consequently, seasonal changes in conditions and herd management should be taken into consideration when evaluating MUN data.

From the public health point of view urea, added as adulterated item has a cancerous effect on the human system and can lead to gradual impairment of the body as suggested by a recent report of Indian Council of Medical research (ICMR) (**Dean, 1985** and **Fox, 1992**).

Major problem associated with excess urea happens when kidneys begin to work overtime to rid the body of excess urea, they also excrete large amounts of minerals, the most important of their components is calcium. The high concentration of calcium mixed with uric acid in the kidneys form kidney stones and the body becomes depleted of calcium and draws it out of the bones rendering them weak, the ending result can be osteoporosis (**Atukorale, 1979**).

In essence, urea is a waste product plays a very important role in that it helps set up the countercurrent system in the nephrons. The countercurrent system in the nephrons allows for reabsorption of water and critical ions (**Walter, 2003**).

Excess of urea in milk on boiling decomposes to carbonic acid, acetic acid, and ammonia, those ammonia-containing calculi might be formed by the partial fermentation of urea in the bladder Also, it may convert into biuret which causes fall in blood pressure and produces strong irritation in the urinary tract (**Prout, 2003**).

As a final point, more researches will however be carried out for finding the effects of other factors like different heat treatment measures, processing and storage.

CONCLUSION AND RECOMMENDATION

It can be concluded from the present investigation that analysis of cow's raw milk present as bulk tank milk, sold in Damietta governorate indicates that all examined samples were healthy and not polluted with excess urea as adulteration process subsequently, these exhibiting a wide array of hazardous impacts on human health.

In order to protect Egyptian human from the hazard effects of such chemicals the following recommendations should be applied:

- Periodical inspection of markets by inspectors of ministry of health should be in forced to control and to minimize risks of adulteration.
- Health ministry laboratories facilities need to be supplemented and upgrading to be capable of performing all tests related.
- Education programs should be imposed for producers and handlers to avoid addition of chemicals to raw milk and to improve the quality of raw milk and its products to ensure maximum safety to the consumers.
- Consumers are advice to avoid consuming raw milk, if necessary they should avoid purchasing from street vendors and purchase it from dairy shops that are licensed from the health minister offices.
- Egyptian Standards must conclude the permissible limits of chemicals added to increase the shelf life and preservatives of raw milks.
- Intersectoral cooperation with different ministries and nongovernmental organization should be increased.
- Hazard analysis and critical control point (HACCP) system must be applied in dairy field.

REFERENCES

- Atukorale, D.P. (1979):** Dangers of eating animal proteins., Hara Marano (Ed.), New York, 51.
- Bector, B.S.; Ram, M. and Singhal, O.P. (1998):** Rapid platform test for the detection of added urea in milk., *Indian Dairyman*, 50 (4): 59-62.
- Broderick, G.A. and Clayton, M.K. (1997):** A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen., *J. Dairy Science*, 80 (11): 2964–2971.
- Calberry, J. (2003):** Milk urea nitrogen testing to improve protein utilization in dairy cattle., Program Assistant/OMAFRA "Ministry of Agriculture, Food and Rural Affairs" 414/54.
- Carlsson, J. and Bergström, J. (1994):** The diurnal variation of urea in cow's milk and how milk fat content, storage, and preservation affects analysis by a flow injection technique., *Acta Veterinaria Scandinavica* 35 (1): 67–77.
- Carlsson, J. Bergström, J.; and Pehrson, B. (1995):** Variations in breed, age, season, yield, stage of lactation, and herd in the concentration of urea in bulk milk and individual cows' milk., *Acta Veterinaria Scandinavica*, 36 (2): 245–254.
- Cashman, K.D. (2002):** Macrominerals in milk and dairy products, nutritional significance. In: H. Roginski, P.F. Fox and J.W. Fuquay, Editors, *Encyclopedia of Dairy Sciences*, Academic Press, London, UK, pp. 2051–2058.
- Dean, J.A. (1985):** *Lange's handbook of chemistry* (13th Ed.). New York: McGraw-Hill, pp. 3–36.
- DePeters, E.J. and Ferguson, J.D. (1992):** Non-protein nitrogen and protein distribution in the milk of cows., *J. Dairy Science*, 75 (11): 3192–3209.
- FAD "Food & Agriculture Department" (2005):** Quick methods for detection of adulterants/contaminants in common food products., Technical committee, 16/T (1631) C.
- Ferguson, J.D. (2000):** Milk Urea Nitrogen., Center for Animal Health and Productivity., New Bolton Center., 382 West Street Road, Kennett Square, PA 19348-1692, USA.
- Fox, P.F. (1992):** *Advanced Dairy Chemistry.*, London, New York., Chapman & Hall.
- Godden, S.M.; Lissemore, K.D.; Kelton, D.F.; Leslie, K.E.; Walton, J.S. and Lumsden, J.H. (2001):** Factors associated with milk urea concentrations in Ontario dairy cows., *J. Dairy Science*, 84 (1): 107–114.

- Gustafsson, A.H. and Palmquist, D.L. (1993):** Diurnal variation of rumen ammonia, serum urea, and milk urea in cows at high and low yields., *J. Dairy Science*, 76 (2): 475–484.
- Hoppe, C.; Mølgaard, C. and Michaelsen, K.F. (2006):** Cow's Milk and Linear Growth in Industrialized and Developing Countries., *Annual Review of Nutrition* Vol. 26: 131-173.
- Jenkins, D.M.; Delwiche, M.J.; DePeters, E.J. and Bon-Durant, R.H. (2002):** Factors affecting the application of on-line milk urea sensing., *Biological Engineering Division of ASAE*.
- Jonker, J.S.; Kohn, R.A. and Erdman, R.A. (1998):** Using milk urea nitrogen to predict nitrogen excretion and utilization in lactating dairy cows., *J. Dairy Science*, 81 (10): 2681–2692.
- Jonker, J.S.; Kohn, R.A. and High, J. (2002):** Use of milk urea nitrogen to improve cow diets., *J. Dairy Science*, 85:939-946.
- Kohn, R. (2000):** Caution needed when interpreting MUNs., *Hoard's Dairyman*, 145 (2): 58.
- Lacks, S.A (1979):** Properties of urea .*Anal. Biochem*, 100, pp 357-363.
- Payne, J.M. (2001):** Milk Urea Nitrogen test (MUN),, *Allen Young Dairy Extension Specialist*, Utah State University, Logan, Utah., AG – 515.
- Prout, W. (2003):** Preparation and analysis of urea., *American Association for Clinical Chemistry, Inc Clinical Chemistry*, 49: 699-705.
- Renny, E.F.; Daniel, D.K.; Krastanov, A.I. and Zachariah1, C.A.R. (2005):** Enzyme based sensor for detection of urea in milk., *Biotechnol. & Biotechnol. Eq.*
- Rodriguez, L.A.; Stallings, C.C.; Herbein, J.H. and McGilliard, M.L. (1997):** Diurnal variation in milk and plasma urea nitrogen in Holstein and Jersey cows in response to degradable dietary protein and added fat., *J. Dairy Science*, 80 (12): 3368–3376.
- Schonberg, F. (1956):** *Milch-Kunde and Milch hygiene.*, 7. Auflage, Verlag M. and H. Scheber, Hannover.
- Sethi, C.K. (2005):** Unbranded milk unfit for human consumption laced with bacterial water, chemicals., *A Tribune Investigation.*, *Tribune News Service*.
- SPSS (1993):** *Statistical Package for Social Science.*, *SPSS® base 9.0 User's Guide.*, Chicago, IL, USA.
- Walter, F. (2003):** *Medical Physiology: A Cellular and Molecular Approach.*, Elsevier/Saunders, 1300., ISBN 1-4160-2328-3, pp. 837.
- Wolfschoon-Pombo, A. and Klostermeyer, H. (1982):** Die NPN-fraktion der kuhmilch. *Milchwissenschaft*, 37 (1): 10–12.

تقدير اليوريا في اللبن البقري المُباع في محافظة دمياط

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المُلخص العربي

أجريت هذه الدراسة لبيان مدى تواجد مادة اليوريا المُضافة إلى عينات اللبن الخام البقري الطازج، حيث تم إجراء الدراسة على عدد ٥٠ عينة عشوائية، تم تجميعها من الباعة الجائلين ومحلات البقالين ومحلات الألبان المنتشرة بمحافظة دمياط، تم إجراء التحليل للعينات لتقدير مستوى تواجد مادة اليوريا المُضافة للعينات باستخدام الاسبكتروفوتوميتر.

دلت النتائج على تواجد مادة اليوريا في عينات اللبن الخام البقري الطازج بنسبة ١٠٠%، حيث كان متوسط تركيز مادة اليوريا Urea في عينات اللبن الخام البقري الطازج 18.047 ± 0.203 مجم/ديسيلتر. في حين دلت النتائج بعد مُعاملة العينات الإيجابية بالحرارة باستخدام الغليان على تواجد مادة اليوريا Urea في عينات اللبن الخام البقري بمتوسط تركيز 17.44 ± 0.198 مجم/ديسيلتر.

من نتائج هذه الدراسة تبين أنها تقع في نطاق الحد الطبيعي لتواجد اليوريا في اللبن الخام (١٠-١٦ مجم/ديسيلتر) في حين إذا زاد تركيز مادة اليوريا Urea عن (٢٠ مجم/ديسيلتر) يعد ذلك غير طبيعي أي مُضاف، وعليه يكون اللبن الخام البقري المُباع في محافظة دمياط صحي وآمن للاستهلاك الأدمي، هذا وقد تم مناقشة النتائج والأهمية الصحية العامة وما يجب أن يُتبع.