RELATIONSHIP BETWEEN PROTEIN/FAT, FREE AMINO ACIDS AND CASEIN FRACTIONS IN DIFFERENT TYPES OF BULK MILK DURING AS AFFECTED BY SEASONAL VARIATION. Gomaa, M.SH\*; M .E. Abdel-Aziz\* ; and S.I. Salama\*\* \* Dairy Dept. , Faculty of Agric. Mansoura University, Mansoura, Egypt . \*\*Dairy Chemistry Department, Animal Production Research Institute, Sakha, Kafr



El-Sheikh, Ministry of Agriculture, Egypt.

## ABSTRACT

This study was carried out in order to establish a relationship between Protein/fat ratio, free amino acids, and casein fractions of cow, buffaloe and goat milk. The amino acid contents of the examined milks were also detected quantitatively and qualitatively. Fat and protein contents generally increased in milk during the period extended from may until the end of laction period. Aspartic acid and glutamic acid were found to decrease in goat milk, which explains the increase of formalin coefficient. The goat milk characterized with an increase in phenylalanine, lysine and histidine. Casein of buffalo's milk was of higher content of isoleucine, and completely free from lysine, proline, glutamic and serine. Casein of goat and cow relatively contained the same concentration of cysteine, which is not found in buffaloe milk.

Keywords: lactation period - Amino acids- casein

## INTRODUCTION

The natural and composition of milk has been a subject of researches since the earliest days of science. Mach progress has been achieved by the first decade of century due to the new development in analytical methodology resulting in rapid advances toward understand ding of milk and its properties. It is shown that milk protein composition was rather constant throughout the season. The milk fat and protein contents are lower in summer than in winter in a pattern opposite to that of milk yield. The maximum differences between monthly averages of milk fat and protein contents and milk yields were 3 g/kg, 2 g/kg and 2.5 kg/day, respectively Coulon (1994), the effect of the reason is often hidden by the changes due to the stage of lactation. This agrees with other studies that have shown that the casein number and protein composition can only to a small extent be influenced by feeding practices (Coulon et al., 1998). Today it is known that a-CN contains two proteins called aS1- and aS2-CN and that  $\gamma$ -CN is a proteolytic product after cleavage of  $\beta$ -CN by plasmin. Hence, the bovine casein group contains four individual gene products known as αS1-CN, αS2-CN, β-CN and κ-CN in approximate proportions of 4:1:4:1 (w/w). Each of the four main casein components show further variability due to different degree of phosphorylation, glycosylation, disulphide bonding, proteolysis and genetic polymorphism (Ng-Kwai-Hang, 2002).Di sulphide bonds can only occur between cystein or cystin residues, which do not exist in αS1-CN or β-CN. However, aS2-CN contains two cystein residues forming one intermolecular disulfide bridge whereas k-CN contains two free cystein residues (Walstra, Wouters and Geurts, 2006). The aim of this study use to Follow up and monitoring seasonal variations in protein and fat contents in Goat's, cow's and buffalo's milk as well as, finding out the relationships among the milk protein (casein) and free amino acids of goat's, cow's and buffalo's milk.

#### MATERIALS AND METHODS

Goat's, cow's and buffalo's milk was obtained from the herd of Sakha animal Production Research Station, Kafr El-Sheikh., Egypt. Goat's milk samples (45) and cow's milk samples (48) used in the present study were taken from Sakha Experimental Station, Kafr Elshiekh Governorate for chemical analysis. Buffalo's milk samples (48) used in the present study was taken from Mahalet Mouse Experimental Station, Kafr Elshiekh Governorate for chemical analysis.

**Fat content:** The Gerber's method was followed as described by B.S.I, (1952).

Total protein (T.P) was determined by means of Kjeldahl method according to Ling (1963).

**Statistical analysis:** Protein was determined by Kjeldahl method and formol number was also determined for the same samples and by using computer program (Excel 2003). Analysis of variance and Duncan's test as well as average and standard error were carried out using a SPSS computer program (SPSS, 1999).

Amino acids content was assayed after deproteinization with 3.5% sulphosalicylic acid using a Bechman 119CL amino acid analyser according to Mondino (1969).

# **RESULTS AND DISCUSSION**

**Protein/fat ratio in goat's milk throughout the lactation period:** Data in Table (1) and Fig. (1) Showed that protein and fat gradually increased from May up to the end of lactation period. Also, correlation between fat and protein throughout the lactation period was positive. Generally, the protein/fat ratio reached 1.019503 all over the lactation period.

**Protein/fat ratio for cow's milk throughout the milking period:** Data in Table (2) and Fig. (2) showed that fat and protein gradually increased from May up to the end of lactation period. Also, correlation between fat and protein throughout the lactation period was positive. The protein/fat ratio was 0.653367 all over the lactation period.



Fig. (1): Effect of lactation period on protein and fat conents of goat's milk.

Table (1): Protein, fat and	protein/fat ratio of goat's milk	throughout the milki	ng period.
Month of the year	Protein%	Fat%	Protein/f

Month of the year	Protein%	Fat%	Protein/fat ratio
May	3.154	3.610	0.873684
June	3.538	3.458	1.023134
July	3.573	3.248	1.100062
August	4.189	3.877	1.080474
September	4.625	4.523	1.022551
Overall	3.816	3.743	1.019503



Fig. (2): Effect of lactation period on protein and fat contents of cow's milk.

## Gomaa, M.SH. et al.

Table (2): Protein, fat and protein/fat ratio of cow's milk throughout the milking period
---

Month of the year	Protein%	Fat%	Protein/fat ratio
January	2.929	4.875	0.600821
February	2.867	4.170	0.687530
March	2.972	4.345	0.684005
April	2.868	4.215	0.680427
May	2.864	4.305	0.665273
June	3.014	4.465	0.675028
July	3.199	4.835	0.661634
August	3.365	5.375	0.626047
September	3.483	5.470	0.636746
October	3.651	5.650	0.646195
November	3.700	5.825	0.635193
December	3.679	5.735	0.641500
Overall	3.216	4.939	0.653367

**Protein/fat ratio for buffalo's milk throughout the milking period:** Data in Table (3) and Fig. (3) Showed that fat and protein gradually increased from May up to the end of lactation period. Also, correlation between fat

and protein throughout the lactation period was positive. The protein/fat ratio reached 0.50666 all over the lactation period.



Fig. (3): Effect of stage of lactation on protein and fat contents of buffalo's milk.

Table	(3):	Protein.	fat and	protein/fat	ratio c	of buffalo'	s milk	throughout	the milking	period
	<- / ·								· · ·	

Month of the year	Protein%	Fat%	Protein/fat ratio
January	4.057	6.520	0.62224
February	3.755	6.240	0.60176
March	3.356	6.550	0.51237
April	3.480	7.030	0.49502
May	3.400	6.865	0.49526
June	3.483	7.315	0.47614
July	3.672	7.925	0.46334
August	3.498	7.425	0.47111
September	3.898	7.970	0.48908
October	3.949	7.870	0.50178
November	4.054	8.135	0.49834
December	3.923	8.650	0.45353
Overall	3.711	7.3746	0.50666

On the other hand, data in Table (4) showed that free amino acid of goat's milk casein has a lower content of arginine, isoleucine and valine, compared to casein of cow's milk, but richer in histidine, alanine, glutamic and threonine . Casein of buffalo's milk is less in alanine, arginine, lysine, threonine and valine, compared to cow's milk. Goat's milk, howevers doesn't contain amino acid proline . These results are in accordance with those reported by (Fox and McSweeney, 1998) and (Pisano et al., 1994). Protein of goat's milk was the objective of many studies in the prementioned review. More recently, it was reported that goat's milk richer in some amino acids like histidine, threonine, methionine and phenylalanine (Sarkar and Misra, 2006). The formol titration is used for the rapid determination of protein in milk. The method depends on the fact that when formaldehyde is added to neutralised milk, free acid (which can be titrated with alkali) is produced in proportion to the amount of proteins present. The protein content is then obtained by multiplying the titration by an empirical factor, which depends on the ratio of casein to albumin and also the particular technique employed. The method proposed by Pyne (1932). It is possible to estimate the protein content rapidly by means of the formol titration. Although amino acids are too wake to be titrated directly with alkali, if formalin is added the - NH<sub>2</sub> group is replaced by the methylene-imino group -N =CH<sub>2</sub> and the carboxyl group is then available for titration. The reaction is often written as:

R.COOH.NH<sub>2</sub> + H.CHO → H<sub>2</sub>O + R.COOH.N= CH<sub>2</sub>. From here the difference was in the parameter value that we can say that the dairy differ in the concentration and the number of the acidic amino acids which include more than one group of COOH that is terminal like acids Aspartic acid and Glutamic acid. It has been found that goat's milk in many of the research is less in the content of acidic amino acids as Aspartic acid and Glutamic acid 6.3 and 20.3 respectively, and this evidence from the increase of formol factor in goat's milk than cow and buffalo's milk.

Data in Table (5) showed that the values of total casein of goat's, cow's and buffalo's milk were 2.51, 2.54 and 3.79, respectively. The highest value of total casein was 3.79 was recorded in buffalo's milk. Goat's milk case has a lower content of  $\alpha$ -s case (0.65), compared to a-s casein of cow's and buffalo's milk (1.26 and 1.86), respectively. B-casein of goat's milk has a higher content, compared to cow's milk, but β-casein of buffalo's milk was higher than goat's and cow's milk. The highest value of k-casein was recorded in buffalo's milk (0.48), and the lowest value of k-casein was recorded in cow's milk (0.36). Whey protein of buffalo's milk was of higher content ,compared to goat's milk, but whey protein of cow's milk was higher than buffalo's milk. The present data coincided with those mentioned by Jenness (1980) and Walstra, Wouters and Geurts,( 2006).

Amino acid	Goat	Cow	-
Arginine	2.1	4.1	2.8
Cysteine	0.4	0.4	-
Glycine	2.1	2.1	8.6
Lynnine	9.1	10	-
Lysine	3.5	8.1	7.6
Alanine	3.6	3.4	2.4
Aspartic acid	6.3	7.4	7.8
Methionine	6	3.2	2
Proline	-	11.8	-
Glutamic acid	20.3	23.2	-
Histidine	5	3.2	1.6
Tryptophan	1.3	1.5	1.5
Serine	5.2	6.6	-
Valine	5.7	7.5	5.6
Tyrosine	4.8	5.8	4.2
Threonine	5.7	4.3	3.7
Phenylalanine	14.6	5.4	4.5
Isoleucine	4.3	6.6	13.6

Table (5): major protein fractions of goat's, cow's and buffalo's mil
---

Protein	Goat's milk	Cow's milk	Buffalo's milk
Total casein	2.51	2.54	3.79
α-s casein	0.65	1.26	1.86
β-casein	1.28	0.7	1.43
k-casein	0.42	0.36	0.48
Whey protein	0.47	0.8	0.63
α-lactoglobulin	0.28	0.3	0.15
β-lactalbumin	0.08	0.4	0.40
Serum albumin	0.05	0.03	0.03

Gomaa, M.SH. et al.

## REFERENCES

- Coulon, J. B. (1994). Effect of physiological stage and season on the chemical composition of milk and its technological properties. Recueil- de-Medecine-Veterinaire 170:6-7, 367-374.
- Coulon, J. B.; Hurtaud, C.; Remond, B. and Verite, R. (1998). Factors contributing to variation in the proportion of casein in cow's milk true protein: a review of recent INRA experiments. Journal of Dairy Research, 65: 375-387.
- Fox, P.F. and McSweeney, P.L.H. (1998). Dairy chemistry and biochemistry. Blackie Academic & Professional. London.
- Jenness, R. (1980). Composition and characteristics of goat milk: Review 1968-1979. J. Dairy Sci., 63: 1605-1630.
- Ling, E. R. (1963). A text Book of Dairy Chemistry Vol. 2. 3rd Ed., Chapman and Hall, London UK.
- Mondino, A. (1969). A new system of automatic amino acid analysis. J. Chromatography, 39:262.

- Ng-Kwai-Hang, K.F. (2002). Milk proteins; Heterogeneity, fractionation and isolation. In Encyclopedia of dairy sciences. Edited by H. Roginski, J.W. Fuquay & P.F. Fox. Academic press. London. 1881-1902.
- Pisano, A.; Packer, N.H.; Redmond, J.W.; Williams, K.L. and Gooley, A.A. (1994). Characterization of O-linked glycosylation motifs in the glycopeptide domain of bovine κ-casein. Glycobiology 4, 837-844. 51
- Pyne, G. T. (1932). The formol titration of milk. Biochem. J. 26: 1005, 27: 915.
- SPSS (1999). SPSS for windows. Release 10.0 (27 Oct. 1999). Standard version. Copyright SPSS Inc., 1989-1999.
- Sarkar, S. and Misra, A. K. (2006). Potential of goat milk for yoghurt manufacture. A review. Indian J. Dairy Sci. 59: 271.
- Walstra, P.; Wouters, J.T.M. and Geurts, T.J. (2006). Dairy Science and Technology. Taylor & Francis. New York.

العلاقة بين البروتين / نسبة الدهون والأحماض الأمينية الحرة وشقوق الكازين في الالبـان المختلفـه خـلال موسم الحلابه.

> محمد شلبي جمعه\*، محمد الدسوقي عبد العزيز محمد\* و سمر ابراهيم سلامه \*\* \* جامعه المنصوره -كليه الزراعه – قسم الالبان جمهوريه مصر العربيه

\*\*معهد بحوث الانتاج الحيواني -مركز البحوث الزراعيه- قسم كمياء الالبان – سخا كفر الشيخ

أجريت هذه الدراسة من أجل إقامة علاقة بين نسبة البروتين / الدهون والأحماض الأمينية الحرة،وشقوق الكازين فى اللبن الجاموسى و البقرى ولبن الماعز . تم الكشف عن محتويات الأحماض الأمينية كميا ونوعيا.و عموما محتوى الدهن و البروتين يزيد من شهر مايو وحتى نهايه موسم الحلابه . حمض الأسبارتيك وحمض الجلوتاميك وجد منخفض فى نسبته فى اللبن الماعز وهو ما يفسر زيادة معامل الفورمالين. وجد ان اللبن الماعز مرتفع فى محتواه من حمض الفينيل ألانين، ليسين و الهستادين . ووجد ان كازين اللبن الماعز وهو ما يفسر زيادة معامل الفورمالين. وجد ان اللبن تماما من ليسين، البرولين، الجلوتاميك وسين ليسين و الهستادين . ووجد ان كازين اللبن الجاموسى مرتفع فى محتواه من الموتاس وحدى السين و خالى وماما من ليسين، البرولين، الجلوتاميك وسيرين. بينما كازين اللبن الماعز و هو ما يفسر نيادة معامل الفورمالين. والسين و والي تماما من ليسين البرولين، الجلوتاميك وسيرين. بينما كازين اللبن الماعز و البقرى يحتوى تقريبا على نفس الموسي من الم