SHORT NOTE [NOTA CORTA]

Tropical and Subtropical Agroecosystems

BIOLOGICAL VALUE OF GOAT MILK CASEIN

[VALOR BIOLÓGICO DE LA CASEÍNA DE LA LECHE DE CABRA]

Samir A. Salem; Elsayed I. El-Agamy; Fatma A. Salama and Nagwa H. Abo-Soliman

Department of Dairy Science, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt. <u>samirsalem51@yahoo.com</u>, <u>eielagamy@yahoo.com</u> *Corresponding author

SUMMARY

The effect of feeding of goat and cow milk caseins on the body weight gain, body organs, erythrocytic & leukocytic counts and their parameters, plasma lipid profile, liver enzyme activities, renal function and plasma proteins of rats over a period of 45 days was studied. Feeding of goat or cow milk caseins had no significant effect on the parameters studied (P \leq 0.05) between rats fed either milk. However, rats fed on goat milk casein showed a significant increase in high density lipoproteins, which are considered more useful, and a decrease in low density lipoprotein in blood of rats fed on goat milk casein.

Key words: Biological value- goat milk casein

INTRODUCTION

Milk is an established basic food and represents one of the most complete single food within human diet .Milk proteins have been used as references for evaluation of the nutritive value of food protein (FAO/WHO, 1973). This is essentially due to the fact that they seem to be the only ones originally intended to function as a single source of nutrients for the offspring. However, it is obvious that there is quite a difference in the protein composition and thereby also the amino acid composition between milks obtained from various species. Meanwhile, the use of milk proteins in non dairy products is of increasing interest as they can be supported as an alternative to other animal or vegetable proteins due to their high nutritional value. Most of the nutritional studies on milk proteins dealt with cow and human milks (Hambraeus); however some other studies on buffalo (Shamsia et al, 2008), camel (El-Agamy (2006) were carried out. The present study is a trail to evaluate the nutritive value of casein prepared from milk of Egyptian goat breed and compare it with that of cow milk.

MATERIALS AND METHODS

Milk

Cow and goat milk samples were collected from the herds of the Faculty of Agriculture, Alexandria University, Egypt.

Animals

Male white albino rats (about 48.0 ± 9.0 g) were obtained from the Animal Breeding Colony of the High Institute of Public Health, Alexandria University, Egypt.

Casein preparation

The whole casein was prepared from raw skim-milk by slow acidification with 0.1N HCl to pH 4.6 at 25 \degree C (Warner, 1944).

Alkaline native-polyacrylamide gel electrophoresis (Alkaline native- PAGE)

Serum proteins were separated on 7.5%T polyacrylamide gel electrophoresis in the absence of SDS and β -mercaptoethanol and the discontinuous buffer system (Hames and Rickwood, 1990). Serum was diluted 1: 20 with the sample buffer (0.0625M Tris-HCl, pH 6.8, 10% glycerol and 0.002% bromophenol blue). After gel polymerization, 20µg protein were applied to each lane in the gel. The was performed electrophoresis using Mini-PROTEAN II cell (Bio-Rad) at 75V through stacking gel followed by 125V to the end of electrophoresis (2hr). After electrophoresis gels were stained for 30 min using 0.1% Coomassie blue R-250 (Bio-Rad) and then distained using a distaining solution of glacial acetic acid, methanol and water (1: 4: 9).

Hematological assays

The concentration of hemoglobin (Hb) was assayed by the method of Wintrobe (1965) using commercial kits Egle diagnostics,. Total erythrocytes count (TEC) was counted according to method of Heplar (1966). Total leukocytes count (TLC) and Deferential leukocytes count were determined according to England and Bain (1976). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from (Hct), TEC and Hb.

Lipids and lipoprotein assays

The cholesterol, triglycerides and high density lipoprotein (HDL) were determined by the methods of Watson (1960), Fossati and Principe (1982) and Warnick et al. (1983), respectively.

Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated according to the methods of Bergmenyer (1985) and Warnick et al. (1983), respectively.

LDL = Cholesterol - [HDL + VLDL]VLDL = Triglyceride $\div 5$

Plasma protein assays

Albumin concentration was determined according to the method of Doumas et al. (1977). Globulin concentration was determined by subtracting the albumin concentration from total protein concentration (Doumas et al., 1977) and albumin/globulin ratio (A/G) was calculated.

Liver function tests

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Total bilirubin was determined by the method of Jendrassik et al. (1938).

Renal function tests

Urea determination was performed according to the method of Patton and Crouch (1977). Creatinine concentration was determined according to the method of Houot et al. (1985).

Animals housing and feeding

Rats were fed in animal houses at Faculty of Agriculture (Damanhour), Alexandria University. Rats were kept under normal health conditions and fed on the experimental diets consisted of 20% goat-milk casein or cow's milk casein (control) (protein \geq 85%), 5% soybean oil, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 10% sucrose, 0.2% choline bitartarate, 55.3% corn starch (Reeves et al., 1993). Water and diet were provided *ad libitum*. Rats were divided randomly into 2 groups (n = 5)

according to the following scheme: group 1 which fed on cow-milk casein; group 2: which fed on goatmilk casein. During the experimental period (45 days) rats were kept separately in well aerated cages $40 \times 24 \times 20$ cm. The diet consumed and body weight was recorded every week of the experimental period. By the end of the experimental period all feed was removed. Blood samples were taken from all rats for analyses. The animals were slaughtered and internal organs of each rat (liver, kidney, spleen, heart, brain, lung and testes) were removed and their corresponding weights were recorded.

Statistical analysis

Results were analyzed using analysis of variance of the SAS package (SAS, 1985).

RESULTS & DISCUSSION

Table (1) presents the effect of feeding of goat and cow caseins on the body weight of rats over a period of 45 days. There was a significant difference between goat and cow milk caseins in the rats weight gain . the weight gain was higher in case of goat casein than that of cow casein. Meanwhile, the effect of feeding of goat and cow caseins to rats on the different body organs was also recorded. There were no significant differences between the two groups were noticed (Table 2).

Table (3) presents the distribution of erythrocytic count and its parameters in blood of rats fed on goat and cow casein. No significant differences between the two groups in all parameters measured were found. Similar results were recorded with leukocytic counts (Table 4).

It was reported that in a nutrition trial involving 38 children, one-half of them were fed goat milk and the other half fed cow milk for 5 months. Results revealed that children in goat milk group surpassed those on cow milk in weight gain, stature, skeletal mineralization, bone density, blood plasma, vitamin A, calcium, thiamine, riboflavin, niacin and hemoglobin concentrations and minimal differences in blood hemoglobin, various other biochemical and structural measurements between the two groups were found (Park, 2006).

Plasma lipid profile

It is well known that animal proteins such as casein, especially prepared from cow's milk, are more hypercholesterolemic than other plant proteins when fed to rats in low fat, cholesterol-free diets (Carroll and Kurowska, 1995). Table (5) shows the distribution of plasma lipids of rats fed on goat and cow casein. Since our experiments were based on feeding rats casein-containing diets, so it is expected that the hypercholesterolemic phenomena will be appeared in serum of rats fed diets contained cow's milk casein. When this level of serum cholesterol was compared to that due to the effect of goat casein, the results showed that there was no significant difference between cow and goat casein, i. e., the same effect was observed.

No significant differences between the two groups were recorded in triglycerides and very low-density lipoproteins; however, there was a significant (P \leq 0.05) decrease in low density lipoprotein concentration in blood serum of rats fed on goat casein. On the contrary, there was a significant (P \leq 0.05) increase in high density lipoprotein in the serum of goat-casein fed rats compared to that of cow casein.

It is well known that very low-density lipoproteins (VLDL_s) are very large complexes contain about 90% lipid, 50-65% of which is triglycerides. VLDLs are synthesized primarily in the liver and serve to transport triglycerides from the liver to other tissues, especially to adipose tissue. Low-density lipoproteins (LDL_s) are served as cholesteryl ester transport because most of the cholesterol contained in the blood plasma of a normal human after an overnight fast is present in LDL_S and about of 75% of cholesterol in LDL is in the form of cholesteryl esters. A single apoprotein comprises 98% of the protein present in LDL. LDL_s are formed in the plasma during catabolism of VLDL. Moreover, high-density lipoproteins (HDL_s) are synthesized in the liver and intestine. They act as catalysts, facilitating the catabolism of VLDL. They serve in removal of excess tissues cholesterol from and lipoproteins (Montgomery et al., 1990).

It was found that there is an inverse relation between decreased HDL and high prevalence of coronary heart diseases (CHD). HDL less than 35 mg/dl is associated with major risk of CHD (Paul, 1991). Other investigators showed that feeding rats on cow casein or whole milk had no effect on the cholesterol concentration in serum of rats of each group (Marquez-Ruiz et al., 1992). It is found that cow milk casein is more hypercholesterolemic than other plant proteins when fed to rats in low fat, cholesterol-free diets (Carroll and Kurowska, 1995).

Serum proteins:

Table (6) presents the distribution of serum proteins in sera of rats fed on goat and cow caseins. Results showed that blood sera of rats fed on goat casein had significantly ($P \le 0.05$) increase in total protein due to the higher ratio of globulins than that of cow casein. This may explain the higher rate of weight gain of rats fed on goat casein (Table 1). The results revealed that no significant difference in blood serum albumin concentration of both groups.

The changes in blood serum proteins of rats fed on goat or cow casein were monitored by alkaline-native PAGE (Fig. 2) .The electrophoretic patterns of individual serum showed that albumin (Alb) was the major band in all sera of goat and cow fed rats. The intensity of Alb band differed markedly among individuals in goat casein group comparing to those of cow casein. It was obvious that the intensity of IgG, transferrin as well as alpha and beta globulins was higher in sera of rats fed on goat casein than that of cow casein. The higher the intensity of IgG in sera of goat casein group may be considered a bioindicator to the humoral immune system stimulator.

Liver enzyme activities

Table (7) presents the distribution of liver-enzyme activities and total bilirubin concentration in plasma of rats fed on goat or cow casein. There was no significant differences observed in the activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) as well as total bilirubin concentration in plasma of both groups.

Renal function tests

Results presented in Table (8) show the distribution of renal function tests of rats fed on goat and cow caseins. No significant differences in urea and creatinine in blood of rats fed on either goat or cow casein.

Conclusion

On the bases of the obtained findings, it can be concluded that goat and cow-milk caseins almost have the same influences biological value except that goat milk casein was characterized by a significant influence on increasing both body weight gain and high- density lipoproteins as well as decreasing Lowdensity lipoproteins.

REFERENCES

- Bergmeyer, H.U. 1985. Methods of enzymatic analysis. 3rd ed., VIII pp. 154-160.
- Carroll, K.K., Kurowska, E.M. 1995. Soy consumption and cholesterol reduction: Review of animal and human studies. J. Nut. 125, 594S - 597S.

Salem et al., 2009

- Doumas, B.T.; Watson, W.A., Biggs, H.G. 1977. Albumin standards and measurement of serum albumin with bromocresol green. Clinica Chimica Acta, 31, 87-96.
- El-Agamy, E.I. 2006. Camel milk. In: Park, Y.W. & Haenlein, G.F. Eds. Handbook of milk of non-bovine mammals. Blackwell publishing Ltd, USA. pp. 297-344.
- England, J.M., Bain, B.J. 1976. Total differential leukocyte count. Brit. J. Haematol. 33, 1-7.
- FAO/WHO, 1973. Protein and energy requirements. WHO Technical Report Series, No. 522. WHO, Geneva.
- Fossati, P., Principe, L. 1982. Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chem. 28, 2077-2080.
- Hambraeus, L. 1992. Nutritional aspects of milk proteins. Fox, P.F., Ed. Advanced dairy chemistry -1: Proteins . Elsevier Applied Science, London, pp. 457-490.
- Hames, B.D., Rickwood, D. 1990. Gel electrophoresis of protein: A practical approach. TRL, England Publishing Co., London. pp. 34-48.
- Heplar, O.E. 1966. Manual of Clinical Laboratory Method. Thomas, Spring Field, Illinois, USA.
- Houot, O., Siest, C.O., Heny, J., Scile, F., Young, D.S. 1985. Interpretation of clinical laboratory tests. pp 220-234.
- Jendrassik, L., Grof, P. 1938. Vereinfachte photometrische methoden zur bestimmung des blutbilirubin. Biochemistry. Z. 297, 81-89.
- Marquez-Ruiz, G., Richter, B.D., Schneeman, B.O. 1992. Modification of triacylglycerides and apolipoprotein B in rats fed diets containing whole milk, skim milk and milk proteins. J. Nut. 122, 1840-1846.

- Montgomery, R., Conway, T.W., Spector, A.A. 1990. Lipoproteins. In: Biochemistry. A caseoriented approach, the C.V. Mosby Co., St. Louis, p. 680.
- Park, Y.W. 2006. Goat milk. Chemistry and nutrition. In: Park, Y.W. and Haenlein, G.F. Eds. Handbook of milk of non-bovine mammals. Blackwell Publishing Ltd, USA. pp. 34-58.
- Patton, G.J., Crouch, S.R. 1977. Colorimetric method for the determination of serum urea. Analyt. Chem. 49, 464-469.
- Reeves, P.G., Roossow, K.L., Lanlauf, J. 1993. Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. J. Nut. 123, 1923-1931.
- Reitman, S., Frankel, S.A. 1957. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American J. Clinic. Path. 28, 56-63.
- Shamsia, S.M., El-Agamy, E.I., El-Ghanam, M., Zina, H., 2008. Buffalo milk proteins. I. Immunological characterization. Biovision Alexandria Conference, April, 11-14, Bibliotheca Alexandrina, Alexandria, Egypt
- SAS 1985. User's guide: Statistics, version 5, Cary, NC, USA: SAS Inst.
- Warner, R.C. 1944. Separation of α- and β-casein. J. Am. Chem. Soc. 66. 1725-1731.
- Warnick, G.R., Benderson, V., Albers, N. 1983. Selected Methods. Clinic. Chem. 10, 91-104.
- Watson, D.A. 1960. Sample method for the determination of serum cholesterol. . Clinica Chimica Acta. 5, 589-594.
- Wintrobe, M.M. 1965. Clinical Hematology, 4th ed. Lea and Febiger, Philadelphia. U.S.A.

Group	Initial weight (g)	Final weight (g)	Weight gain (%)
Cow casein	48.38 ± 9.71^a	56.86 ± 11.21^{a}	17.53 ± 0.84^a
Goat casein	48.63 ± 9.27^a	59.44 ± 11.16^{a}	22.23 ± 1.80^{b}

Table 1. Effect of feeding of cow and goat caseins on the body weight of rats over a 45 -days period (Mean \pm SE).

Means in each column having different superscript letters are significantly different at $P \le 0.05$.

Table 2. Effect of feeding of cow and goat caseins on the relative organ weights (%) (Mean values ± SE).

Organ	Cow casein (N=5)	Goat casein (N=5)
Final body weight (g) (100%)	56.86 ± 11.21^{a}	59.44 ± 11.16^{a}
Brain (%)	2.81 ± 0.28^{a}	3.03 ± 0.56^{a}
Heart (%)	0.48 ± 0.02^{a}	0.54 ± 0.03^{a}
Lung (%)	1.33 ± 0.36^{a}	$1.24\pm0.16^{\rm a}$
Liver (%)	4.90 ± 0.30^{a}	5.16 ± 0.36^a
Spleen (%)	0.41 ± 0.03^{a}	$0.49\pm0.07^{\rm a}$
Kidney (%)	$1.38\pm0.08^{\rm a}$	1.33 ± 0.11^{a}
Testes (%)	1.16 ± 0.19^{a}	0.71 ± 0.20^{a}

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$.

Table 3. Distribution of erythrocytic count and its parameters in blood of rats fed on cow and goat caseins (Mean \pm SE).

Parameters	Cow casein (N=5)	Goat casein (N=5)
TEC ($\times 10^{6}$ /ml)	8.47 ± 0.38^a	$8.85\pm0.18^{\rm a}$
Hb (g/dl)	14.46 ± 0.47^{a}	$14.68\pm0.41^{\rm a}$
MCH (Pg)	17.16 ± 0.43^{a}	$16.50 \pm 0.34^{\rm a}$
MCHC (g/dl)	36.76 ± 0.42^a	36.06 ± 0.37^{a}
MCV (fl)	46.58 ± 0.71^{a}	45.68 ± 0.81^{a}

TEC = Total erythrocytic count.

Hb = Hemoglobin.

MCH = Mean corpuscular hemoglobin.

MCHC = Mean corpuscular hemoglobin concentration.

MCV = Mean corpuscular volume.

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$.

Parameters	Cow casein (N=5)	Goat casein (N=5)
TLC ($\times 10^3$ /ml)	9.48 ± 0.21^{a}	8.50 ± 0.73^{a}
Neutrophils (%)	48.20 ± 1.98^{a}	47.80 ± 0.86^a
Eosinophils (%)	3.00 ± 0.32^{a}	2.60 ± 0.24^{a}
Basophils (%)	0.00	0.00
Lymphocytes (%)	46.00 ± 1.82^{a}	$47.00\pm0.89^{\rm a}$
Monocytes (%)	$2.80\pm0.20^{\rm a}$	$2.60\pm0.24^{\rm a}$

Table 4. Distribution of leuckocytic count and its parameters in blood of rats fed on cow and goat caseins (Mean \pm SE).

TLC = Total leukocytes count.

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$.

Table 5. Distribution of	nlasma linide of rate fo	d on cow and goat case	ing (Maan values \pm SF)
radic J. Distribution of	plasma nplus of fats ic	u on cow and goat case	ms (whether values $\pm 5E$).

Parameters	Cow casein (N=5)	Goat casein (N=5)
Triglycerides (mg/dl)	$144.28 \pm 14.55^{\rm a}$	152.97 ± 14.97^{a}
Cholesterol (mg/dl)	115.60 ± 8.24^{a}	121.85 ± 7.10^{a}
LDL (mg/dl)	37.89 ± 2.99^{a}	$22.57\pm0.85^{\text{b}}$
HDL (mg/dl)	48.67 ± 2.35^{a}	$68.69 \pm 4.48^{ m b}$
VLDL (mg/dl)	28.86 ± 2.91^a	30.59 ± 2.99^{a}
LDL/HDL (ratio)	$0.78 \pm 0.02^{ m a}$	0.33 ± 0.03^{b}

LDL = Low - density lipoprotein.

HDL = High - density lipoprotein.

VLDL = Very low - density lipoprotein.

LDL/HDL ratio = Low - density lipoprotein/High - density lipoprotein.

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$.

Table 6. Distribution of serum p	proteins of rats fed on cow and g	goat caseins (Mean values \pm SE).
----------------------------------	-----------------------------------	--------------------------------------

Parameters	Cow casein (N=5)	Goat casein (N=5)
Total protein (g/dl)	$9.19\pm0.18^{\rm a}$	$10.22\pm0.22^{\rm b}$
Albumin (g/dl)	$5.24\pm0.20^{\rm a}$	$5.79\pm0.20^{\rm a}$
Globulin (g/dl)	3.95 ± 0.03^{a}	4.43 ± 0.02^{b}
A/G (ratio)	$1.32\pm0.06^{\rm a}$	1.30 ± 0.04^a

A/G = Albumin/Globulin ratio.

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$.

Parameters	Cow casein (N=5)	Goat casein (N=5)
AST (U/L)	51.06 ± 2.98^{a}	52.18 ± 3.79^a
ALT (U/L)	34.34 ± 1.18^{a}	33.12 ± 2.56^a
Total bilirubin (mg/dl)	0.59 ± 0.07^a	$0.54\pm0.08^{\rm a}$

Table 7. Distribution of liver-enzyme activities of rats fed on cow and goat caseins (Mean \pm SE).

AST = Aspartate amino transferase.

ALT = Alanine amino transferase.

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$.

Table 8. Distribution of renal function tests of rats fed on cow and goat caseins (Mean values \pm SE)		
	estribution of renal function tests of rate fed on cow and goat caseins	$(M_{OOD} v_{OD} v_{OD} + SE)$
1000000000000000000000000000000000000	surbution of renar function tests of rats red on cow and goat caseins	(which wanted \pm SE).

Parameters	Cow casein (N=5)	Goat casein (N=5)
Urea (mg/dl)	68.25 ± 0.69^{a}	64.77 ± 2.34^a
Creatinine (mg/dl)	$1.86\pm0.28^{\rm a}$	2.09 ± 0.26^a

Means in each row having the same superscript letters are not significantly different at $P \le 0.05$



Figure 2. Alkaline native –PAGE (pH 8.8, 10%T) of rat serum proteins. a: Lanes 1 - 5: Individual serum samples of rats fed on goat casein; b: Lanes 1-5: Individual serum samples of rats fed on cow casein; IgG: Immunoglobulin G; Anode is toward bottom of photo.

Submitted June 27, 2008 – Accepted May 13, 2009