ships among strains M 20, RGA and Mankarso is indicated.

Summary. Serological and cultural studies for leptospirosis were conducted on 37 opossums trapped at a military reservation at Aberdeen, Md. Five of the 37 animals were serologically positive. Questionable reactions were seen in serums from 9 other animals. Two isolates were obtained and identified as *Leptospira ballum* and *L. icterohaemorrhagiae,* respectively. *L. icterohaemorrhagiae* in an opossum had not heretofore been reported. The antigenic interrelationships among the "complete" and "incomplete" biotypes of *L. icterohaemorrhagiae* and *L. mankarso,* disclosed in the serologic studies, differed from previously reported findings.

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Amino Acid Composition and Nutritive Value of Goat Milk Casein.* (27441)

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Much attention has been directed to the amino acid composition of various proteins and to the relationship between amino acid composition and nutritive value. However, there is relatively little information on the casein of goat milk. This is surprising in view of the extensive literature(1,2,3,4) on the casein of cow's milk and the frequent use of goat milk as a therapeutic agent.

The information available concerning the amino acid composition of goat milk was published in 1933(5,6) and was obtained by fractional solubility and steam distillation technics. No values are available for glycine, isoleucine, methionine, or threonine. Published values for arginine and histidine vary greatly.

In this study, we have calculated the nutritive value of goat milk casein by the rat growth method of Miller and Bender(7) and have compared it with that of cow's milk casein. The same preparation of goat milk casein was separated into its component amino acids by ion exchange chromatography by the method of Moore and Stein(8) and measured by quantitative ninhydrin assay(9).

Experimental. I. *Preparation of casein.* Commercially available evaporated goat milk† was employed as a source of casein. Casein was isolated by isoelectric precipitation, as described by Jacobs(10). It was redissolved and reprecipitated 2 more times before use. The final precipitate was filtered on coarse filter paper and dried *in vacuo* at 50°C. The dried protein was extracted 3 times with boiling diethyl ether and dried *in vacuo* at room temperature. It was then ground to a very fine powder in a Wiley Mill and used for rat

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† Kindly supplied by California Goat Dairymen's Assn. Inc.

TABLE I. Composition of Diets (% by Weight).

	Diet 1	2	3	4
Goat milk casein	0	10	0	0
Cow's " "	0	0	10	0
Vitamin-free casein	0	0	0	10
Cottonseed oil	10	10	10	10
Powdered sucrose	80.8	70.8	70.8	70.8
Cellulose	3	3	3	3
Salt mixture (USP XIV)*	4	4	4	4
Vitamin mix†	2.2	2.2	2.2	2.2

* Hawk, Oser, and Summerson(14).

† This yielded the following amounts of vitamins in g/kg of diet: thiamin .022, riboflavin .022, nicotinic acid .100, calcium pantothenate 0.65, pyridoxine .022, inositol .110, choline chloride .170, vitamin A 20,000 units, irradiated ergosterol 210,000 units, α-tocopherol .110, ascorbic acid .100, biotin .00045, folic acid .0020, cyanocobalamin .00003.

growth studies and for amino acid analyses. Cow's milk casein controls were prepared from commercially available evaporated cow's milk in the same manner. A sample of commercially available vitamin-free casein‡ was also tested.

II. *Determination of nutritive value.* The biological values of goat milk casein and cow's milk casein, prepared as described above, and of commercial vitamin-free casein, were determined according to the method of Bender(11). Composition of the diets is shown in Table I.

Long-Evans rats weighing 50 to 60 g each obtained from an established commercial dealer were divided into 4 diet groups of 8 animals each. They were selected so that all groups had approximately equal mean weights and equal weight distributions. The animals were housed in individual cages with screen bottoms and were given the prepared diet and water *ad libitum* for 10 days. On the tenth day the uneaten food was weighed and food consumption calculated. The animals were weighed and then sacrificed by etherization.

The carcasses were dried to constant weight *in vacuo,* then extracted with ether on a Soxhlet apparatus for 18 hours. The fat-extracted remains were digested in 200 ml of 20% H_2SO_4 at 15 lb pressure for 18 hours. The digests were diluted to standard volume and the nitrogen analyzed by a semi-micro Kjeldahl procedure(12). Each carcass was analyzed individually. From these data the body composition was calculated in terms of fat, water, and protein.

‡ Nutritional Biochemicals Corp.

During the experiment, all feces were collected and total fecal nitrogen excretion of each animal was determined by the Kjeldahl procedure. The values were used for the calculation of the digestibility factor. Nitrogen content of each diet was measured by the Kjeldahl procedure.

III. *Amino acid composition.* A portion of goat milk casein, the same preparation as that incorporated in the diet, was used for amino acid analysis. Nitrogen content was determined by the Kjeldahl procedure.

Approximately 100 mg of protein was hydrolyzed with 20 ml of 6N HCl for 20 hours at 115°C. The hydrolysate was filtered and the precipitate was washed with water. The filtrate and the wash water were combined and dried *in vacuo* at room temperature. This was repeated a third time. The amino acid mixture was then made up to a standard volume with distilled water. The amino acids were separated on Dowex 50-X8 resin§ according to the method of Moore and Stein(8). Approximately 3 mg of protein hydrolysate was used for each column. Quantitative measurement of the effluent was carried out by the Ninhydrin method of Rosen(9). To determine sulfur containing amino acids, a portion of the casein was oxidized with performic acid as described by Hirs(13), then hydrolyzed and chromatographed as described above. The amount of cystine found was too small to accurately determine.

Results and discussion. In Table II are

TABLE II. Weights and Food Consumption of Rats Fed 4 Diets.

Group	Initial wt	Final wt	Diet eaten	Wt change g diet
	(all values in g)			
Non-Protein	54±3	47±3	62±14	−.123±.035
Goat Milk Casein	61±5	77±8	81±15	.198±.057
Cow Milk Casein	61±2	80±6	88± 9	.216±.080
Vitamin-free Casein	59±4	79±4	83± 8.5	.245±.055

Means and standard deviations are given.

§ Obtained from Bio-radiation Laboratories, Berkeley, Calif.

TABLE III. Body Composition of Rats on 4 Diets.

Diet	Fat, % of total body wt	Water, % fat-free wt	Protein, % fat-free wt
Non-Protein	5.7±1.1	75.7±1.5	14.8±2.1
Goat Milk casein (10%)	7.4±2.5	78.6±1.4	14.5±1.0
Cow Milk casein (10%)	3.7±2.0	76.7±2.3	14.8±1.8
Vitamin-free casein (10%)	5.4±1.7	77.0±1.4	15.3±1.2

Means and standard deviations are given.

shown average initial and final weights of rats fed the 4 diets, average quantity of food eaten by each group, and weight gained per gram of food eaten. Slightly more weight was gained by the animals fed either of the cow's milk casein preparations than was gained by the animals consuming goat milk casein. The weight increase per gram of food was considerably greater for the animals eating vitamin-free casein than for the animals in either of the other 2 groups.

Table III shows the body composition of rats fed the 4 diets. There were variations in the amount of total body fat but the total water and protein, when expressed in terms of fat-free body weight, remained constant in the 4 groups of animals. This is in agreement with Bender's(11) observation that only total body water need be measured, as the ratio of protein to water at any age is constant. However, we have used the experimentally determined values for nitrogen throughout our calculations, rather than assume a particular protein to water ratio.

Table IV shows the net protein utilization, net protein ratio, protein retention efficiency, digestibility and biological value of the 4 diets, determined from the following equations:

I. Net Protein Utilization (NPU) $= \frac{B - (B_K - I_K)}{I}$.

Where B is total body nitrogen on protein diet, B_K is total body nitrogen on non-protein diet, I is intake of nitrogen on diet, I_K is intake of nitrogen on non-protein diet.

II. Net Protein Ratio $= \frac{\text{Grams protein gained}}{\text{Grams protein eaten}}$.

III. Protein retention efficiency = Net Protein Ratio × 16.

IV. Digestibility (D) $= \frac{\text{Grams N eaten} - \text{grams N excreted}}{\text{Grams N eaten}}$.

V. Biological Value $= \frac{\text{NPU}}{\text{D}}$.

It is seen that goat milk casein has growth promoting activity and biological value similar to that of commercially obtained vitamin-free cow's milk casein but both proteins have lower biological values and net protein utilization than does cow's milk casein prepared as described above. On the basis of net protein ratio there is little difference among the 3 caseins tested, and they all are equally digestible. One should note that these nutritional evaluations are determined at limiting levels of dietary protein.

Table V shows the amino acid composition of goat milk casein as compared with published values for goat, cow and human casein. The primary differences between goat and cow casein are a) the larger quantity of glycine in goat milk casein as compared with cow's milk casein, b) the lower concentration of arginine in goat milk casein, and c) the lower concentration of sulfur-containing amino acids, particularly methionine, in goat milk. The relative deficiency of methionine may account for the lower biological value of goat milk casein but we have not investigated this by feeding goat milk casein diets supplemented with methionine.

The concentrations of the other 2 amino acids not previously reported in goat milk casein, isoleucine and threonine, are similar to concentrations of these amino acids in cow's milk casein.

Rauschning's(6) suggestion that anemia following ingestion of goat milk casein is due to a deficiency of histidine is not supported

TABLE IV. Nutritive Value of Goat Milk Casein and Cow Milk Casein.

Group	NPU	NPR	PRE	D	BV
II Goat Milk Casein	66%	3.9	62.5%	89.5	73.8
III Cow " "	79%	4.0	64.0%	91.0	86.8
IV Vitamin-free Casein	68%	3.7	59.0%	89.5	76.0

NPU = Net protein utilization, NPR = Net protein ratio, PRE = Protein retention efficiency, D = Digestibility, and BV = Biological value.

TABLE V. Amino Acid Composition of Caseins (g/100 g protein).

Amino acid	Goat milk Casein (this investigation)	Goat milk* casein (avg)	Cow milk* casein (avg)	Human milk* casein
Alanine	2.65	1.8	2.3	2.0
Arginine	1.34	2.66 (1.32 & 4.0)	3.76	3.43
Aspartic Acid	7.62	1.7	5.8	4.6
Cystine	Trace	Trace	0.34	0.64
Glutamic Acid	18.27	12.1	21.7	20.9
Glycine	1.41	No data	0.4	0
Histidine	3.65	1.85 (3.3 & 0.4)	2.25	1.77
Isoleucine	5.24	No data	6.0	6.3
Leucine	9.43	11.4	10.8	12.2
Lysine	5.24	3.7	6.85	5.4
Methionine	1.35	—	2.88	2.4
Phenylalanine	3.77	2.0	5.54	5.8
Proline	8.29	6.9	9.8	8.9
Serine	4.10	0.7	5.45	5.4
Threonine	4.38	—	4.35	4.5
Tryptophan	Not determined	1.37	1.22	1.4
Tyrosine	3.22	4.87	5.99	5.7
Valine	6.47	6.0	6.64	5.0

* Data obtained from Macy, Kelly and Sloan(15).

by our results. We found a higher level of histidine in goat milk casein than in cow's milk casein. Lang(5) suggests that Rauschning's low value for histidine may have represented the extremes of variation from sample to sample. Our value for histidine is very similar to that reported by Lang.

Summary. 1. Casein from goat milk was prepared by isoelectric precipitation followed by ether extraction. Amino acid composition was determined by chromatography on Dowex 50 ion exchange resin. The nutritive value of goat milk casein was determined by a rat growth assay method. 2. Goat milk casein has a higher content of glycine and lower concentrations of arginine and methionine than the values reported for cow's milk casein. The earlier reports on goat milk casein did not include values for glycine, isoleucine, methionine or threonine. 3. The biological value of goat milk casein, as prepared in our laboratory, is similar to that of commercially available cow's milk casein. The cow's milk casein prepared in our laboratory, however, had a somewhat higher biological value than either of the other two samples. 4. These differences in amino acid composition may be the basis for the differences in biological value of caseins from goat and cow's milk.

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