16589

Nutritive Value of Casein as Influenced by Alcohol Extraction.*

P. H. Derse, H. Nath,† C. A. Elvehjem, and E. B. Hart.
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Since casein is an important constituent of most purified rations, methods of preparing and purifying this protein are of importance to all nutritionists. Many workers have used alcohol extraction and there is definite evidence that traces of vitamins remaining in crude casein are removed by the procedure. Hartman and Cary[1,2,3,4] found that rats grow at a much slower rate when fed casein thoroughly extracted with 95% alcohol than when fed the untreated casein. They believe that an unknown factor necessary for the normal growth of the rat is removed and they termed the substance factor X, or a factor of palatability. The reduced rate of growth of rats fed the alcohol extracted casein was accompanied by a decreased food consumption. Normal growth was secured by supplementing the alcohol extracted casein ration with certain liver concentrates.

The following study was undertaken in order to determine if similar results could be obtained under our experimental conditions and if the alcohol treatment could have any effect on the release of the amino acids in the treated casein.

Experimental. Pregnant rats of the Sprague-Dawley strain, were placed at parturition on a diet consisting of sucrose or dextrin 55%, alcohol extracted casein 20%, cotton seed oil containing the fat soluble vitamins (Table I) 10%, brewers yeast 10%, and salts IV (Hegsted *et al.*[5]) 5%. When the young from these females were 21 days of age they were placed in individual cages with raised screen floors. Care was taken to ensure equal distribution of the young on the basis of weight, sex, and parentage. Experimental diets and water were fed *ad libitum* and individual body weights were recorded weekly over a 5 week period. Composition of the rations and the total weight gains are given in Table I.

The extracted casein was prepared by boiling 2.5 kg of crude casein with 4 liters of 95% ethanol for 8 hours with continuous stirring. This procedure was repeated 10 successive times. It is evident from Table I, Experiments 1 and 2, that a definite decrease in rate of growth occurred in all the male rats receiving extracted casein as compared to those receiving the ration containing crude casein. The female rats showed a much smaller difference.

For the following experiments the extraction of the casein with ethanol was increased to 10-24 hour periods, and dextrin was used as the carbohydrate. With this more thoroughly extracted casein greater differences were observed with both male and female rats. When the protein was increased to 40% of the ration, the difference was somewhat less but could be attributed to the reduced rate of growth of the rats fed the high level of crude casein. Likewise, when the yeast supplement was replaced by synthetic vitamins, the rate of growth over the 5 week experimental period was decreased significantly when

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by grants from the Evaporated Milk Association, Chicago, and the National Dairy Council, Chicago, in behalf of the American Dairy Association.

† Government of India Research Fellow.

1 Cary, C. A., Hartman, A. M., Dryden, L. P., and Likely, G. D., *Fed. Proc.*, 1946, **5**, 128.

2 Cary, C. A., and Hartman, A. M., *Fed. Proc.*, 1946, **5**, 137.

3 Cary, C. A., and Hartman, A. M., *J. Am. Diet. Assn.*, 1946, **22**, 1016.

4 Cary, C. A., and Hartman, A. M., *U. S. Dept. Agr. Pub.*, Sept., 1947.

5 Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1940, **138**, 459.

TABLE I.
Composition of Rations* and Growth Rate of Rats.

Exp. No.	Group	Crude casein	Extracted casein	Sucrose	Dextrose	Dextrin	Brewers yeast	Vit.†	Supplement	5-wk wt gain‡ Male g	5-wk wt gain‡ Female g
1	1		20		**55**		**10**			150	114
	2	20			55		10			175	118
	3		25	60				x		158	117
	4	25		60				x		176	123
2	1		20		55		**10**			159	115
	2	20			**55**		10			—	124
	3		25	60				x		114	80
	4	25		60				x		148	117
3	1		20			55	10			85	85
	2	20				55	10			166	122
	3		40			35	10			103	64
	4	40				35	10			149	104
	5		40	45				x		107	74
	6	40		45				x		122	92
4	1		20			55	10			121	93
	2		20			55	10	x		123	108
	3		20			55	10		Crude concentrate	111	78
5	1		20			55	10			135	118
	2	20				55	10			188	131
6	1		20			55	10			127	108
	2		20			55	10		Purified concentrate	124	81
	3	20				55	10			185	106
7	1		20			65		x		148	114
	2		20			65		x	Liver powder§	154	120
	3	20				65		x		197	132
	4	20				65		x	" "	188	140
8	1		20			65		x		141	100
	2		20			65		x	Fresh liver	177	134
	3	20				65		x		190	138

* All rations contained salts IV 5%, and vitamin supplemented cottonseed oil 10%. 500 g of cottonseed oil was supplemented with 7.52 mg 2-Me-1,4-naphthoquinone, 0.502 mg calciferol, 20.0 mg β-carotene, and 1.08 mg α-tocopherol.

† Each 100 g of ration contained 0.50 mg thiamin, 0.60 mg pyridoxine, 0.70 mg riboflavin, 3.00 mg calcium pantothenate, 150 mg choline hydrochloride, 0.60 mg nicotinic acid, 0.025 mg biotin, 0.20 mg folic acid, 15.00 mg *p*-aminobenzoic acid and 50.0 mg inositol.

‡ Each figure represents the average weight of 3 animals.

§ Wilson solubilized liver powder (Fraction "L").

TABLE II.
Amino Acid Content of Raw and Alcohol Extracted Casein After Hydrolysis with Acid or Enzymes.

	Acid hydrolysis*		Enzyme hydrolysis†	
	Raw	Alcohol extracted	Raw	Alcohol extracted
Arginine	3.54	3.27	4.48	4.11
Aspartic acid	6.50	6.82	0.3	0.5
Cystine	0.20	0.17	0.08	0.06
Glutamic acid	20.1	20.9	11.0	9.75
Histidine	2.46	2.55	1.14	1.04
Isoleucine	5.05	5.26	5.93	4.09
Leucine	9.0	9.15	8.82	8.77
Lysine	6.45	6.35	4.69	4.7
Phenylalanine	4.77	5.1	4.43	4.27
Proline	10.1	10.3	3.70	3.60
Threonine	3.87	4.25	3.48	3.36
Tryptophan	1.05	1.16	1.13	1.08
Tyrosine	4.1	4.25	4.08	4.6
Valine	6.45	6.92	5.86	5.54
α-Amino nitrogen‡	100	100	53	55

* Casein was autoclaved for 10 hours with 25 vol. of 6 N HCl at 15 lbs pressure, except in the case of tryptophan, where 20 vol. of 5 N NaOH was used and autoclaved for 5 hours at 15 lbs pressure.

† 1 g of casein was digested with 100 mg pancreatin (Merck + 20 mg hog intestinal mucosa (Wilson), with shaking at 37°C for 3 days at pH 8-6, after making the volume to 100 ml.

‡ Per cent liberation of α-amino nitrogen (Van Slyke) based upon complete liberation of amino groups after acid hydrolysis.

the extracted casein was used.

Rats ingesting the more completely extracted casein developed a characteristic hair loss on the dorsal, anterior and posterior areas of the body. This hair loss gradually enveloped the entire dorsal region of the animal.

The alcohol filtrates were reduced to a volume of 200 cc and used as a crude concentrate. A more purified concentrate was prepared by allowing the crude filtrate to stand in the cold (-4° C) for 24 hours, filtering and drying the precipitates at 65° C.

The addition of either the crude or the purified alcohol concentrate from casein at a level equivalent to 20% casein (Experiments 4 and 6) failed to enhance growth but did prevent hair loss. In all cases, the use of crude unextracted casein increased the rate of growth. The addition of fresh liver at the rate of one gram per rat per day brought the rate of growth of the rats receiving extracted casein to normal and also prevented hair loss. Solubilized liver powder at a level of 2% in the ration did not affect the growth rate or hair loss.

In order to determine if the reduced nutritive value of the casein was due to the loss of or change in any of the constituent amino acids, the raw and extracted casein were analyzed for 14 different amino acids using the method of Henderson and Snell.[6] In one series the determinations were made after acid hydrolysis and in the other series enzymatic digestion was used in order to detect any possible difference in the rate of release. The results are given in Table II and it is evident that the values for the 2 caseins are almost identical. Methionine values are not recorded because the standard curve was not acceptable. However, there was no difference in the response of the test organism to the hydrolysates prepared from the two caseins. The values for aspartic acid and glutamic acid in the enzyme hydrolysates may not be true values since no attempt was made to determine asparagine and glutamine. Since not all the amino acids especially serine were studied it is still possible that changes occur in certain amino acids which are responsible for the observed difference in nutritive value. It is also possible that the *in vitro* methods used do not give a true indication of the *in vivo*

[6] Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, **172**, 15.

release of amino acids from the two caseins.

If the greater nutritive value of crude casein is due to an impurity, this growth promoting factor must be fairly heat stable since it is present in crude casein which is heated during its preparation. However, after extraction with alcohol and concentration of the extracts, the activity cannot be recovered. The fact that the extract prevents hair loss indicates that either a partial loss of the factor occurred during concentration or that two factors are involved—one with prevention of hair loss and one with growth.

It is also important to point out that the alcohol treatment used may produce toxic substances which appear in both the extracted casein and the alcohol extracts. The fact that similar results were obtained with sucrose, dextrose and dextrin and the fact that the addition of the concentrates gave further weight retardation, give added weight to this possibility. Food consumption records also showed a decreased food consumption associated with the reduced growth. If the effect is due to the presence of a toxic factor, the addition of fresh liver overcomes this action either by counteracting the toxicity or by supplying factors which cannot be produced by intestinal flora in the presence of the toxic material.

Summary. The nutritive quality of crude casein is reduced by continuous extraction with hot 95% ethanol. These results confirm the observations of Hartman and Cary. Rats fed rations containing such extracted casein grow at a reduced rate and develop a characteristic hair loss. The alcohol extracts obtained from the extraction of crude casein do not increase the rate of growth of rats ingesting extracted casein but do prevent the characteristic hair loss. The growth rate of rats ingesting alcohol extracted casein can be increased to normal by feeding fresh liver. The analysis of the extracted casein and the crude casein for 14 amino acids following enzymatic or acid hydrolysis failed to show any appreciable differences.

16590

Some Histological Manifestations of a Tryptophan Deficiency in the Chick.

S. O. BROWN, M. C. WILKENING,* AND B. S. SCHWEIGERT.†

From the Departments of Biology and Biochemistry and Nutrition, Agricultural and Mechanical College of Texas, College Station.

Although many investigations have been conducted on the effect of amino acid deficiencies on the growth rate and on the amino acid composition of tissues and excreta, few studies have been conducted on the histological changes in the various tissues attributable to the ingestion of amino acid deficient diets by the animal. Particular attention however, has been given to changes in blood composition, primarily in the level of hemoglobin and plasma proteins.[1] Other studies have revealed corneal vascularization resulting from various amino acid deficiencies[2,3] and atrophy of the thymus, adrenal cortex, liver and epithelial cells of the seminiferous tubules when leucine deficient diets were fed to rats.[4] Similar studies have been conducted

* Present address, Research Laboratories, Security Mills, Inc., Knoxville, Tenn.

† Present address, Division of Biochemistry and Nutrition, American Meat Institute Foundation, University of Chicago, Chicago, Ill.

1 Cannon, P. R., *Advances in Protein Chemistry*, 1945, **2**, 135.

2 Sydenstricker, V. P., Schmidt, H. L., and Hall, W. K., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 59.

3 Sydenstricker, V. P., Hall, W. K., Bowles, L. L., and Schmidt, H. L., *J. Nutrition*, 1947, **34**, 481.

4 Maun, M. E., Cahill, W. M., and Davis, R. M., *Arch. Path.*, 1945, **40**, 173.