Factors Affecting the Nutritive Value of Cow's Milk

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Experiments were conducted with albino rats to study the effect of season, pasteurization, further heat treatment, and other modifications upon the nutritive value of cow's milk. Weanling rats maintained on a cow's milk diet for 6 weeks grew well, and females maintained for a longer period of time reproduced and lactated normally through three generations. No seasonal variation was observed in the nutritive value of raw, pasteurized, or homogenized milk, and neither pasteurization nor homogenization had an adverse effect upon its nutritive value when growth, reproduction, and lactation performance were used as the criteria of measurement. Young rats, however, maintained on a mineralized cow milk diet had a tendency to accumulate excess fat in their livers. This fatty liver condition was found to be directly related to the butterfat content of the milk diet, but was not related to season, pasteurization, or a deficiency of lipotropic factors.

MILK HAS LONG BEEN RECOGNIZED as a food of excellent biological value, and its importance in the diet has been established. Probably no other single food product has received more detailed study and therefore it is impossible to attempt a complete literature review in this report.

It has been demonstrated that raw milk supplemented with iron, copper, and manganese as the sole source of food is adequate for normal growth and reproduction in albino rats. Evans and Phillips (*5*) were able to raise rats through five generations, and Anderson, Elvehjem, and Gonce (*1*) were able to maintain dogs through two generations on a mineralized raw milk diet. The adequacy of milk diets, however, has not been so extensively studied in other animal species.

The effect of pasteurization on the nutritive value of milk has been the subject of considerable investigation and controversy. Extensive studies have been carried out by Henry and Kon (*7*), Krauss (*12*), Brannon and Prucha (*3*), Anderson, Elvehjem, and Gonce (*1*), Richardson and Hogan (*18*), The National Institute for Research in Dairying, Reading, England (*13*), and many others, which indicate that pasteurization has little or no effect upon nutritive value. Pottenger (*14, 15*) and Pottenger and Simonsen (*16*), however, using the cat as an experimental animal report that pasteurization and other heat treatments have a deleterious effect upon the nutritive value of milk and have recommended the feeding of certified raw milk to infants and children.

Elvehjem *et al.* (*4*), Kohler *et al.* (*8–11*), Randle *et al.* (*17*), Sober *et al.* (*21*), and Stirn *et al.* (*22*), using rats and guinea pigs as experimental animals, demonstrated a seasonal change in the nutritive value of cow's milk. As time elapsed since the cows were on fresh pasture, the nutritive value of their milk declined. Pasteurization caused a significant decrease in nutritive value in the case of milk which was seasonally deficient, but had little or no effect upon milk of higher nutritive value. Kohler *et al.* (*8*) postulated a growth factor, "grass juice factor," that was either low or missing in winter milk or milk from cows on dry feed but was present in ample quantities in the milk of cows on fresh grass feed. The factor could be supplied to a deficient milk by supplementation with the pressed juice from fresh grass.

Wulzen and Bahrs (*25*) reported better growth and development in guinea pigs on a mineralized whole raw milk diet than on a pasteurized or skim milk diet. On the latter diets the animals develop a stiffness of the limbs, followed by emaciation, weakness, and finally death. An "antistiffness" factor was postulated which was isolated and reported later to be fat-soluble and heat-labile (*19, 23–25*).

In view of the rather contradictory nature of several important factors concerning the nutritive value of cow's milk, further study was initiated in an effort to extend and clarify earlier observations. The study reported here was conducted with albino rats and represents 18 separate experiments extending over 2 years. The primary objectives of these experiments were (1) to study the effect of season, pasteurization, and further heat treatment upon the nutritive value of cow's milk, and (2) to demonstrate the effect of various modifications of a cow's milk diet upon growth and general well-being.

Studies of Growth and Liver Fat

Male weanling albino rats weighing 40 to 45 grams, from the Sprague-Dawley Co., Madison, Wis., were used as experimental animals. They were housed in individual screen-bottomed cages in an air-conditioned animal room.

The various milk diets were mineralized with 3.0 mg. of iron, 0.15 mg. of copper, and 0.15 mg. of manganese per 100 ml. of milk from iron pyrophosphate, copper sulfate, and manganous sulfate, respectively, and fed ad libitum twice daily.

At the termination of an experiment (in most cases 6 weeks) the animals were sacrificed and the livers removed for fat analysis. Liver fat was determined by the wet ether extraction technique using the Mojonnier flask as outlined by Bixby *et al.* (*2*).

Each experiment contained animal control groups of raw milk, pasteurized milk, and a stock ration. The stock ration had the following composition:

Ground wheat	28
Yellow corn	24
Soybean oil meal	10
Linseed oil meal	10
Alfalfa meal	8
Dried skim milk	12
Wilson's liver concentrate	1
$CaCO_3$	1
Iodized NaCl	1
Melted butter	5

Unless otherwise stated, the milk used was grade A containing about 3.5% butterfat and was obtained three times weekly from a pooled supply from the University of Wisconsin Department of

Dairy and Food Industries. Milk was pasteurized in a laboratory pasteurizer at 143° F. for 30 minutes.

The fresh milks processed outside the city of Madison were received twice weekly by refrigerated truck from the various sources. Bacteriological, phosphatase, and fat determinations were conducted to confirm the freshness and quality of these milks.

Reproduction Study

Two groups of female weanling rats from the Sprague-Dawley Co. were used as the first generation for this investigation. One group was fed exclusively mineralized raw milk from the University Dairy and the other group was fed the same milk pasteurized at 143° F. for 30 minutes. The animals were continued on these mineralized milk diets for approximately 100 days, at which time they were mated with adult males of the Sprague-Dawley strain that had been raised on stock ration for breeding purposes.

When a sharp increase in weight was noted in a female during the gestation period, she was isolated in a large individual cage. The young were counted as soon as possible after birth, but not handled until 24 hours later. They were then weighed, and if necessary, reduced to six per litter. The young were weaned 22 days after birth. The female was continued on the same milk diet, and given several weeks' rest period before mating again. This study was continued until each female of each generation had raised two litters and until three generations of rats had been raised. A growth study employing diets of mineralized raw and pasteurized milk was also conducted with groups of weanling young from each generation.

Results

The results presented in Table I represent pooled data from all experiments with milk from the Department of Dairy and Food Industries. The average mean weekly gain in weight during the 6-week test period for each milk diet is given along with the average mean per cent of liver fat values calculated on the wet basis for each milk diet at the termination of the 6-week test period.

These results indicate that the growth obtained on the milk diets was good but slightly less than that obtained on a stock diet. No difference in growth rate was observed among raw, pasteurized, and homogenized milk which all stemmed from the same original source. No seasonal variation in growth was noticed in either raw or pasteurized milk. The observed variation in growth rate of animals fed the same type of milk diet from one experiment to the next is that which can be normally expected with this type of experimental animal.

The results in Table I further indicate that all the animals on the milk diets at the end of the 6-week test have a liver fat accumulation that is higher than that found in a weanling rat or one that has been fed stock ration. The average liver fat value for a stock ration-fed rat is 4.3%, while that for a milk-fed animal is 7.2%. There is no significant difference between liver fat values of rats fed raw and pasteurized milk, but the values are slightly lower for animals fed homogenized milk. The data presented in regard to seasonal variation and effect on the liver fat accumulation are rather limited, but no significant differences are noted.

Considerable variation has been observed in liver fat values of rats fed the same type of milk among the individual experiments. This variation could be related to unknown differences in the composition of the milk, but are more likely related to the physiological and nutritional status of the animal when received for test.

The results presented in Table II represent the effect of adding various supplementary factors to a milk diet on rat growth rate and liver fat accumulation. Supplementing with choline, folic acid, vitamin B_{12}, methionine, lysine, threonine, or glycine had little or no effect upon growth rate or liver fat accumulation. Addition of magnesium had no effect upon liver fat but slightly depressed growth rate. Supplementing with a mixture of choline, folic acid, and vitamin B_{12} resulted in a slightly lower liver fat value with no effect on growth rate.

The results in Table II indicate further that if the solids content of the milk is increased by the addition of a dried partially delactosed skim milk product or sucrose, the growth rate is little affected, but the liver fat values are slightly decreased. If the butterfat of the milk is removed and replaced with corn oil, the growth rate is depressed

Table I. Weekly Gain and Liver Fat of Albino Rats Fed Mineralized Raw, Pasteurized, and Homogenized Milk Diets

Nature of Milk Diet	No. of Groups[a]	Av. Weekly Gain[b], Grams	Av. Liver Fat[b], %
Average of all milk tests	20	32.8 ± 0.6	7.1 ± 1.0
Effect of milk treatment			
Raw milk tests	8	32.5 ± 0.9	7.8 ± 0.5
Pasteurized milk tests	6	32.0 ± 0.9	7.1 ± 0.6
Homogenized milk tests	6	33.7 ± 1.0	6.0 ± 0.2
Effect of season			
Milk from cows on pasture	11	32.3 ± 0.8	7.5 ± 0.4
Milk from cows on dry winter feed	9	33.4 ± 0.9	6.5 ± 0.4
Raw milk			
From cows on pasture	6	32.8 ± 1.0	7.7 ± 0.7
From cows on dry winter feed	2	33.5 ± 2.5	8.2 ± 0.0
Pasteurized milk			
From cows on pasture	3	30.3 ± 0.9	8.1 ± 0.8
From cows on dry winter feed	3	33.7 ± 1.5	6.1 ± 0.0
Homogenized milk			
From cows on pasture	2	33.5 ± 1.0	6.0 ± 0.6
From cows on dry winter feed	4	33.8 ± 1.8	6.0 ± 0.3
Stock ration	11	35.2 ± 0.9	4.3 ± 0.2

[a] Each group represents six animals on 6-week test.
[b] Each value represents average mean of groups ± standard error of mean of groups.

Table II. Weekly Gain and Liver Fat of Male Albino Rats Fed Mineralized Cow's Milk, Supplemented with Various Factors

(6 animals per group)

Supplemental Factor per 100 Ml. Milk	Av. Weekly Gain, Grams			% Liver Fat, Wet Wt.		
	Supplemented group	Milk control group	Stock control group	Supplemented group	Milk control group	Stock control group
Choline, 27.4 mg.	34.5	34.7	37.2	5.9	6.2	4.1
Choline, 54.8 mg.	33.0	34.0	36.5	6.3	6.9	4.1
Folic acid, 500 γ; Vitamin B_{12}, 3 γ; Choline, 54.8 mg.	33.7	34.0	36.5	5.1	6.9	4.1
Methionine, 60.0 mg.	36.8	34.7	37.2	5.9	6.2	4.1
Lysine, 100.0 mg.	38.2	34.7	37.2	6.7	6.2	4.1
Threonine, 50.0 mg.	32.0	31.8	29.0	5.4	5.5	4.6
Threonine, 50.0 mg.; Choline, 50.0 mg.; Methionine, 50.0 mg.	31.8	31.8	29.0	5.3	5.5	4.6
Magnesium, 48.0 mg.	28.8	34.0	37.2	7.6	8.4	4.8
Magnesium, 24.0 mg.	32.7	34.0	37.2	8.0	8.4	4.8
Sucrose, 10%	34.0	34.0	36.3	5.3	5.8	4.3
Low lactose, 10% dried skim milk	33.0	34.5	30.7	5.2	6.5	4.3

and the liver fat value is slightly increased.

The results presented in Table III indicate the effect of the butterfat content of milk and fresh market milk from diverse sources upon rat growth rate and liver fat accumulation. Milks varying in fat content from 1.0 to 4.75% were prepared by the addition of a proportionate amount of homogenized cream to pasteurized skim milk.

These results indicate that the greater the fat content of the milk, the greater is the accumulation of fat in the livers of the rats after the 6-week test period. Feeding a 1.0% fat milk resulted in an average liver fat value of 4.9%, while a 4.75% fat milk resulted in an average liver fat value of 9.8%. Approximately normal growth was obtained at all fat levels except the 1.0%, where the rate was probably depressed because of the high lactose content and severe conditions of diarrhea. It is apparent that the growth rate of weanling rats on milk does not vary greatly with the source of the milk and that the amount of liver fat accumulation depends more upon the fat content of the milk than its source.

The results presented in Table IV are considered preliminary in nature, to show the effect of heat treatment and processing upon rate of rat growth and accumulation of liver fat. In this experiment regular pasteurized, homogenized milk was subjected to further heat treatments at 145°, 165°, and 185° F.

The 145° and 165° F. heat treatments given the milk did not affect the growth rates, but the 185° F. treatment depressed the growth rate slightly. The further heat treatment had no significant effect upon the accumulation of liver fat.

Dried whole milk was also tested by feeding both dry and reconstituted. In both cases excellent growth resulted, with low liver fat values. A canned sterilized whole milk product also resulted in an excellent growth rate and a low liver fat value.

The data presented in Table V are the results of the study of reproduction and lactation. There is no significant difference between the reproductive and lactation performance of groups of female rats fed raw and pasteurized milk for three generations. This performance was just as good during the second and third generations as in the first. The first litter weanling weights are slightly lower than the others, but this is a common occurrence with a young female.

The results presented in Table VI are the average weekly growth rates for 6 weeks of young weanlings fed raw and pasteurized milk in the reproduction of three generations. There is no difference in the growth rate of young from the animals fed raw milk as compared with young from the animals fed pasteurized milk. There is, however, a significant decrease in the growth rate of the second and third generation on the milk diets as compared with the first generation animals.

Table III. Weekly Gain and Liver Fat of Male Albino Rats Fed Mineralized Cow Milk Diets of Varying Butterfat Content

Type of Milk Diet	*% Butterfat*	*Av. Weekly Gain, Grams*	*Av. % Liver Fat, Wet Wt.*
University Dairy milk	1.00	27.2	4.9
	2.25	32.8	6.0
	3.00	33.7	5.8
	3.50	34.8	6.7
	4.00	38.3	7.0
	4.75	32.5	9.8
Certified milk			
Raw (Guernsey)	4.75	29.2	10.6
Pasteurized (Guernsey)	4.75	30.2	10.7
Pasteurized (Holstein)	4.10	30.8	7.3
Chicago market milk			
Producer A (pasteurized)	3.50	33.2	6.9
Producer B (pasteurized)	3.60	32.8	6.9
Producer A (homogenized)	3.60	33.2	6.2
Producer B (homogenized)	3.50	35.0	6.3

Table IV. Weekly Gain and Liver Fat of Male Albino Rats Fed Mineralized Heat-Treated Cow's Milk

(6 animals per group)

Type of Milk Diet	*Av. Weekly Gain, Grams*	*Av. % Liver Fat, Wet Wt.*
University Dairy milk		
Homogenized	34.5	6.5
Homogenized + heat 145° F., 30 min.	33.0	5.3
Homogenized + heat 165° F., 30 min.	34.8	5.4
Homogenized + heat 185° F., 30 min.	30.7	5.9
Dried whole milk		
Reconstituted	37.3	4.9
Dry	37.7	4.9
Sterilized whole milk, high temperature short-time process	37.0	4.9

Discussion

The results of this study presented in Table I indicate that neither pasteurization nor homogenization has a deleterious effect upon the nutritive value of cow's milk when the criterion of measurement is the growth of weanling rats fed the milk as the sole source of food except for iron, copper, and manganese supplementation. Weanling albino rats grow well when reared on a mineralized milk diet. No seasonal variation in the nutritive value of either raw or pasteurized milk was observed.

These results differ from the findings of Elvehjem *et al.* (*4*) and Kohler *et al.* (*8–11*), who found that summer milk had a higher nutritive value than winter milk and that pasteurization had an adverse effect upon the nutritive value of winter milk but not on summer milk. The only apparent explanation for this difference is that milk produced today may be higher in nutritive value than it was 20 years ago. Knowledge of plant and animal nutrition has expanded greatly in this time, and as a result the dairy cow receives a more complete diet today. This fact could very well reflect in the production of a milk of higher nutritive value today as compared with 20 years ago. And then too, one cannot ignore the possibility that different climatic conditions may account for these differences in results.

It also is evident from Table I that young weanling rats reared on a mineralized milk diet develop a fatty liver condition that cannot be related to pasteurization or season. Homogenized milk gives slightly lower liver fat values, which is probably due to less butterfat consumption because of a lack of creaming in the food dishes.

At first it was thought that a milk diet might be limiting in certain lipotropic factors. A number of these were tested, and it was found that the supplementation of milk with choline, folic acid, vitamin B_{12}, methionine, threonine, lysine, and magnesium had little or no effect upon the accumulation of liver fat. A combination of choline, folic acid, and vitamin B_{12} lowered the liver fat values slightly but hardly enough to be significant. The fat accumulation in the livers of rats on a milk diet, therefore, is not related to a deficiency of these factors in the diet. In this respect the fatty liver condition observed in rats on milk diets differs from the fatty liver

Table V. Reproductive and Lactation Performance of Female Rats Fed Mineralized, Raw, and Pasteurized Cow's Milk for Three Generations

Generation and Type of Milk Diet	No. of Females	% Having Young	% Young Alive		% Young Weaned and Kept	Av. Wt. of Young, Grams, after	
			First observed	After 24 hours		First day	Weaning 22 days
First generation, first litter							
Raw milk	8	88	84	68	78	6.1	25
Pasteurized milk	8	100	84	81	70	6.2	29
First generation, second litter							
Raw milk	5	80	77	56	82	5.6	35
Pasteurized milk	6	83	87	83	85	6.0	39
Second generation, first litter							
Raw milk	6	100	80	80	100	6.4	36
Pasteurized milk	6	100	89	83	83	6.8	29
Second generation, second litter							
Raw milk	5	100	74	71	80	5.9	38
Pasteurized milk	6	100	100	84	87	6.0	34
Third generation, first litter							
Raw milk	6	100	98	96	94	6.2	36
Pasteurized milk	6	100	87	81	82	5.8	37

condition observed by Harper *et al.* (*6*) and Singal *et al.* (*20*) in rats on a 9% casein ration. These workers found that a threonine addition to their basal diet was effective in reducing the accumulation of liver fat.

The possibility that the high butterfat content of milk might be related to the fatty liver condition suggested itself when homogenized milk and milk with added sucrose and milk protein gave slightly lower liver fat values. These additions actually lowered the fat content of the diet. The results presented in Table III clearly indicate that the butterfat content of the milk is directly related to the accumulation of fat in the liver. There is also little doubt that the high liver fat values obtained on the certified milk diets are directly related to their high butterfat content.

The results reported concerning the effect of heat treatment on biological value and liver fat accumulation are only preliminary in nature. Further study is necessary before any definite conclusions can be drawn.

Dried whole milk and a canned sterilized whole milk product both produced excellent growth rates in rats. Certainly the effect of processing in these products had little or no deleterious effect upon the nutritional value of the milk. An interesting observation in testing these processed milks is that all of them resulted in a significantly lower liver fat accumulation than did the fresh milks studied. Because these processed products, however, did not all stem from the same source of fresh milk, it is not possible to make direct comparisons between the results obtained.

It is known that pregnancy and lactation are critical physiological functions and may put even more stress on the organism than growth. This can in some cases provide a more useful measure of an adequate diet than does growth. A diet adequate for optimum performance over one generation may become deficient during succeeding generations. This technique of investigation was, therefore, employed to compare the nutritive value of raw and pasteurized cow's milk.

Table VI. Growth Rate of Weanling Rats from First, Second, and Third Generation Females Receiving Mineralized Milk Diets

Generation and Type of Milk Diet	No. of Animals	Av. Weekly Gain, Grams
First generation, Sprague Dawley		
Male		
Raw milk	8	29.5
Pasteurized milk	8	30.0
Female		
Raw milk	8	22.7
Pasteurized milk	8	22.2
Second generation		
Male		
Raw milk	6	24.0
Pasteurized milk	6	26.0
Female		
Raw milk	6	13.7
Pasteurized milk	6	11.7
Third generation		
Male		
Raw milk	6	26.0
Pasteurized milk	6	23.7
Female		
Raw milk	6	17.7
Pasteurized milk	6	17.5

From the results presented in Table V it can be seen that there is no significant difference between the reproductive and lactation performance of groups of females fed raw and pasteurized milk for three generations. This performance is just as good in the second and third generations as in the first. It can be concluded that the pasteurization of milk does not destroy factors in the milk that are necessary for normal reproduction and lactation in the albino rat.

The results presented in Table VI, however, indicate that young weaned from second and third generation females do not grow as well on a milk diet as the young of first generation females. Higher weanling weights were observed in young from the second and third generations, but whether this is related to these growth differences would require further study with more animals.

As the studies reported here were conducted with the albino rat, there is still a possibility that pasteurization and season could affect the nutritive value of cow's milk for other animal species. The rat is a very versatile animal, in that it can readily adapt itself to many different types of diet. This is not true for many other animal species—for example, the guinea pig is not easily adapted to a milk diet. In a study now in progress the authors have found that a mineralized cow milk diet will not promote optimal growth in this species. The poor growth response of this herbivorous animal to a milk diet may be partly due to the fact that milk is not its natural diet and it, therefore, is not willing to accept it wholeheartedly.

Another animal species employed for studying the adequacy of milk diets is the cat. This animal, as was found with the guinea pig, does not readily adapt itself to a milk diet, and considerable variation has been noted in the willingness to accept the milk as the sole source of food. Several young cats on both a mineralized pasteurized milk diet gained from 1000 to 1500 grams in an 8-month test period, while others would not accept the milk and did not survive. No differences, however, have been noted in the performance of cats fed raw milk diets and pasteurized milk diets.

Summary

Albino rats maintained on a mineralized cow milk diet grow well and reproduce and lactate normally.

No seasonal variation was observed in the nutritive value of raw, pasteurized, or homogenized cow's milk, and neither pasteurization nor homogenization had an adverse effect upon its nutritive value when growth, reproduction, and lactation performance were used as the criteria of measurement.

Weanling rats maintained on a mineralized cow's milk diet have a tendency to accumulate excess fat in their livers. This fatty liver condition was found to be directly related to the butterfat content of the milk diet, and was not related to season, pasteurization, or a deficiency of well-known lipotropic factors in the milk diet.

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COTTONSEED MEAL IN POULTRY FEED

A Distinctive Yolk Component in the Fresh Eggs of Hens Fed Gossypol

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Cottonseed meal is a desirable protein concentrate, but it is not now used in ratios for laying hens because the gossypol it contains has an adverse effect on egg quality, particularly of stored eggs. In this study, an attempt was made to fractionate yolks of normal and "gossypol" eggs to discover differences between them. It was found that yolks of hens fed gossypol contain a yellow component not extractable by acetone, but soluble in 3 to 1 hexane–acetone. Although the amount of this component, estimated by its absorbance at 400 mμ, was related directly to the level of gossypol fed, its absorption spectrum was different from that of gossypol. Quantitative estimation of the egg yolk component will serve as a convenient measure of biologically active gossypol. This work is one step toward obtaining sufficient knowledge to allow use of cottonseed meal in diets of laying hens.

Carefully processed cottonseed meal is recognized as a good source of amino acids for chickens, turkeys, swine, and ruminants (*4*), but it is not at present recommended for use in the rations of laying hens because it may contain enough available gossypol to cause marked discoloration of the egg yolks, especially when the eggs are kept in cold storage. The yolks of such eggs may become reddish or olive colored, and may show considerable mottling. The yolk membrane appears to be thickened, and the yolk material is often gelatinous (*7*).

Schaible, Moore, and Moore (*11*) reported in 1934 that the yolks of broken-out eggs from hens fed cottonseed meal became chocolate brown in color when kept in an atmosphere of ammonia for an hour or less. It has been suggested that this discoloration is related to that which develops during several months of cold storage. Swensen, Fieger, and Upp (*13*) concluded that the discoloration resulted from breakdown, during storage, of tightly bound iron-protein complexes, followed by combination of the iron with gossypol. Despite the plausibility of this theory, little actual evidence has been found to support it. Gossypol has never been isolated from eggs, and its metabolism in the body is not known.

Conflicting reports exist on the advisability of using cottonseed meal in laying rations. One sample of screw-pressed meal was found to be satisfactory at a 20% level, even when the eggs were stored (*12*). Heywang (*5*), however, found that discoloration occurred at free gossypol levels lower than 0.003% of the diet. Individual hens exhibit wide variation in yolk discoloration (*5, 10*).

Before cottonseed meal can be recom-