

RESEARCH PAPERS

**Protein Quality of Stored Dry Skim Milk
With Standard and Lysine-Limiting Diets****M. WOMACK**Nutrition Institute
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Changes in protein quality of dry skim milk stored under conditions where moisture uptake was not a factor were monitored by feeding trials with rats. After 1 yr of storage at 25 or 37 C, no effects on nitrogen digestibility, protein efficiency ratio, or net protein ratio measured by standard procedures were attributable to storage time or temperature. However, when the skim milk samples were incorporated into diets limiting in lysine to study the effect of storage conditions on lysine bioavailability, relative nutritive value decreased by 8% in the sample stored 12 mo at 25 C and by 8, 9, and 8% in samples stored for 3, 6, and 12 mo at 37 C. Chemically available lysine determined by 2,4,6-trinitrobenzenesulfonic acid reagent showed similar small decreases in samples stored at 37 C.

INTRODUCTION

The nutritional deterioration of the proteins of dry skim milk (NDM) during storage under conditions of high relative humidity and high temperature has been documented (5, 8, 13, 15, 16, 20). The decrease in protein quality is due to inactivation of lysine, which, through a Maillard reaction (14, 21), forms an enzyme-resistant compound with lactose; this reaction is favored by a high moisture content in the stored powder (7, 10).

Kramer (19) and others (22) claimed that to

hold loss of protein quality of NDM stored for 1 yr under vacuum in hermetically sealed containers to 10% (measured by protein efficiency ratio (PER)), storage temperature should not exceed 14.5 C. They claimed further that freezer storage at -15 C is needed for retention of 90% of the protein quality when storage for 2 yr is anticipated. Manufacturers of NDM were concerned about these claims because of the high costs of energy for refrigerated and frozen storage.

Clarification of these reports seemed desirable because USDA price support operations often involve extended storage of NDM, sometimes for as long as 2 yr prior to distribution. Therefore, by animal feeding trials with normal diets and diets limiting in lysine we investigated changes in protein quality of NDM stored at moderate and elevated temperatures under conditions where uptake of moisture during storage was not a factor. Concentrations of total lysine and lysine chemically available for reaction with 2,4,6-trinitrobenzenesulfonic acid also were monitored during storage.

MATERIALS AND METHODS

The NDM was a spray-dried commercial product containing 3.3% moisture, 34.9% protein, 7.9% ash, .8% fat, and 53.1% carbohydrate (by difference); it met all requirements for U.S. Extra Grade nonfat dry milk (6). For biological evaluation studies, 2.7 kg samples of NDM were air-packed in No. 10 cans and stored in constant temperature incubators at 25 and 37 C for 6 or 12 mo (25 C) or for 3, 6, and 12 mo (37 C). An additional sample was nitrogen packed and stored at -18 C for 12 mo as a control.

Weanling male albino rats of the Sprague

Dawley strain were obtained commercially and divided into treatment groups of 10 to 12 per group except for 6 per group for a nitrogen-free diet. The animals were housed individually and fed a stock diet for 2 to 3 days after arrival before being assigned to the experimental diets. Average initial weights varied from 61 to 63 g. Food and water were supplied ad libitum. The animal room was maintained at 25 C and 50% relative humidity; it was also light controlled with 12 h periods of light and darkness.

The samples were used in rat feeding trials to study the effects of storage time and temperature on protein quality. In the first feeding trial, nitrogen digestibility (ND), protein efficiency ratio (PER), net protein ratio (NPR), and relative nutritive value (RNV) were determined by standard methods (1, 2). The diets contained sufficient NDM (29.7%) or Animal Nutrition Research Council (ANRC) casein to furnish 10% protein. Other dietary ingredients were (in %) U.S.P. XVIII salt mixture, 5; non-nutritive fiber, 1 (Teklad Mills, Madison, WI)¹; vitamin mixture, 1 (Teklad Mills, Madison, WI)¹; Wesson oil¹, 9.4; and cornstarch to 100%. A protein-free diet in which additional cornstarch replaced the protein source also was prepared. The same lot of casein was used throughout the study.

Weight gains and food intakes were determined weekly. Scattered food was recovered carefully. During wk 2, feces were collected from all rats fed the initial samples; during subsequent evaluations of effects over the storage period, feces were collected from only six animals in each group. Feces were frozen until the end of the collection period, dried under infrared lamps, allowed to equilibrate to ambient moisture, weighed, and ground.

The remainder of the NDM samples not used in feeding trial 1 were stored at -18 C until all samples were at hand and then were used in feeding trial 2. Storage times for these samples were: initial samples, 12 mo; 3-mo samples, 11 mo; 6-mo samples, 7 mo; and 12-mo samples, 1 mo. In feeding trial 2, the amount of lysine in the diets was reduced so that changes in lysine

availability might be detected more readily. The amount of NDM in the diets was reduced from 29.7% to 23.0% and to ensure that no other amino acid was in limited supply, the amino acids in Table 1 were incorporated into the 10% protein diets. Amounts of essential amino acids added were "critical levels" of each amino acid previously established; these critical levels were such that a 20% reduction in any one essential amino acid significantly reduced rat growth (25). The amino acids supplied .36 g nitrogen and the NDM, 1.24 g. Other dietary ingredients were (in %): Jones and Foster salt mixture (17), 4; non-nutritive fiber (Teklad Mills, Madison, WI)¹, 4; vitamin mixture (Teklad Mills, Madison, WI)¹, 1; corn oil, 10; and cornstarch to 100. As before, a protein-free diet also was fed. For convenience, the diets were formulated with 10% protein; because of the small quantity of samples available, diets were diluted with the protein-free diet to 6% protein (N × 6.25) and fed for 21 days to groups of 5 to 6 rats. This technique and variations thereof have been used in a lysine bioavailability study (24) in which slope-ratio assays were reported. Average initial weights of the rats were 61 to 63 g.

Statistical evaluations for significance were by analysis of variance and Student's t test.

Nitrogen was determined in samples and diets by a macro Kjeldahl method (1). Concentrations of total lysine and lysine chemically available for reaction with 2,4,6-trinitrobenzene-

TABLE 1. Amino acids added to dry skim milk diets in feeding trial 2.

	% Diet
Arginine HCl	.20
Cystine	.14
Histidine HCl·H ₂ O	.22
Isoleucine	.32
Leucine	.50
Methionine	.16
Phenylalanine	.30
Threonine	.35
Tryptophan	.09
Tyrosine	.24
Valine	.40
Total	2.912 ^a

¹ Reference to firm or brand name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

^aSupplies .36 g N to the 10% protein basal diets; 1.24 g N supplied by milk sample (total 1.6 g).

TABLE 2. Variation of apparent nitrogen digestibility (ND), protein efficiency ratios (PER), net protein ratio (NPR), and relative nutritive value (RNV) of dry skim milk samples with storage time and temperature (feeding trial 1).

Storage time (mo)	ND ^a (%)		PER				NPR ^b	RNV ^c	
			Observed		Standardized ^d				
	\bar{X}	SE	\bar{X}	SE	\bar{X}	SE	\bar{X}	SE	
Initial	88.4	.63	3.71	.06	2.67	.04	4.13	.05	100
25 C, Air pack									
6	87.3	.38	3.50	.05	2.85	.04	3.96	.05	96
12	84.6	.57	3.60	.06	2.61	.04	4.03	.05	98
37 C, Air pack									
3	88.6	.40	3.53	.09	2.68	.07	4.03	.07	98
6	88.8	.45	3.63	.05	2.96	.04	4.09	.06	99
12	87.2	.48	3.58	.04	2.59	.03	3.99	.04	97
-18 C, N ₂ pack									
12	87.5	.37	3.59	.08	2.60	.06	4.00	.07	97

^a Apparent nitrogen digestibility = (nitrogen intake - fecal nitrogen)100/nitrogen intake.

^b Net protein ratio = (weight loss of group on nitrogen free diet + weight gain)/protein intake.

^c Calculated from NPR.

^d Standardized to 2.50 for casein.

sulfonic acid reagent were measured in the initial sample and in samples stored at 37 C for 3 mo, 5 mo, and 1 yr; samples stored at 25 C and -18 C for 1 yr also were evaluated. Total lysine in the samples was determined by a Beckman model 120 C amino acid analyzer according to the method of Spackman et al. (23). Chemically available lysine was measured colorimetrically as ϵ -TNP-L-lysine by the procedure of Kadake and Liener (18) as modified by Greenberg et al. (11); in that method, reconstituted NDM samples were dialyzed exhaustively and freeze-dried prior to analysis. Total lysine was the average of duplicate analyses on samples hydrolyzed with 5.7 N HCl in sealed evacuated tubes for 24 and 48 h; available lysine was measured in duplicate.

RESULTS AND DISCUSSION

Averages of ND, PER, NPR, and RNV (Trial 1, Table 2) showed no consistent differences attributable to storage time and temperature. Average weight gains of groups of rats fed the seven NDM samples varied from a low of 4.8 \pm .24 g/day (stored 3 mo at 37 C) to a high of 5.9

\pm .24 g/day (stored 12 mo at 37 C) and seemed unrelated to sample treatment. Feed efficiencies (feed efficiency = g weight gain/g feed consumed) showed little variation, ranging from .36 to .38 g/day.

The PERs, corrected to 2.5 for casein, were 2.67 for the initial sample and 2.60 for the nitrogen-packed sample stored 12 mo at -18 C. The average for the air-packed sample stored 12 mo at 37 C was 2.59. Because these were similar, the significantly greater standardized PERs for the samples after 6 mo of storage may have resulted from the unusually low casein PER (3.07 \pm .04) used for standardization. This was significantly ($P = .01$) lower than the other PERs for casein (initial 3.47 \pm .09; 3 mo 3.29 \pm .10; 12 mo 3.45 \pm .05). Therefore, higher standardized PERs for the samples stored for 6 mo should be viewed as an example of the variation in the PER method (12).

In trial 1, the 10% protein diets contained .81 to .86% lysine, calculated from the highs and lows in Table 3. In trial 2 the 10% basal protein diets (N \times 6.25) contained .62 to .66% lysine (see Materials and Methods). When these

TABLE 3. Variation in total and chemically available lysine with storage time and temperature.

Storage time (months)	Total lysine undialyzed	Total lysine dialyzed	Available lysine dialyzed	% Available lysine
	(g/100 g protein)			
Initial	8.32	8.82	8.71	98.8
25 C, air pack 12	8.58	9.18	8.79	95.8
37 C, air pack 3	8.35	9.46	8.76	92.6
5	8.21	9.31	8.53	91.6
12	8.11	9.12	8.13	89.1
-18 C, N ₂ pack 12	8.14	9.66	9.23	95.5

basal diets were diluted to provide 6% protein ($N \times 6.25$), the relative differences in available lysine between diets should have remained unchanged. This conclusion is based on studies using the slope-ratio assay (24) in which NPR's calculated from protein intakes and weight gains that fell on the regression line generally

TABLE 4. Variations of relative nutritive value (RNV) of dry skim milk samples with storage time and temperatures as tested with lysine limiting diets (feeding trial 2).^a

Storage time (mo)	NPR		RNV ^b
	\bar{X}	SE	
Initial	4.99	.12	94
25 C, Air pack 6	5.29	.14	1.00
12	4.86	.09	92
37 C, Air pack 3	4.88	.10	92
6	4.81	.12	91
12	4.85	.11	92
-18 C, N ₂ Pack 12	5.29	.06	100

^a Fed 21 days, 5 to 6 rats/group.

^b Calculated from NPR values.

were similar. For example, the NPR (Table 4) of the NDM sample that was stored 12 mo at -18 C and then fed at 6% protein was $5.29 \pm .06$; NPR's for the same sample fed at 5% and 7% protein were $5.29 \pm .08$ and $5.09 \pm .07$. In actual practice, however, we only compare samples fed at the same protein percent.

Effects related to storage time and temperature seem to be more marked in trial 2 (Table 4). Findings are complicated by the low NPR of the initial sample because this average is not significantly different ($P = .05$) from any of the others. However, NPR's of the sample stored 12 mo at 25 C and of all of the samples stored at 37 C were significantly lower ($P < .01$) than the NPR of the sample stored 12 mo at -18 C under nitrogen. The RNV of these samples (-18 C, 12-mo sample = 100) were 91 to 92. These data suggest that available lysine decreased slightly in samples stored 12 mo at 25 C, that decrease was similar in samples stored 3 mo at 37 C, and that further decrease in availability did not occur during storage up to 12 mo.

Averages for total lysine (Table 3) for the undialyzed samples varied from 97 to 103% of the average for the initial sample, but all were within the error for these analyses. Averages for available lysine as determined chemically decreased in the samples stored at 37 C, but the available lysine of the sample stored 12 mo at 25 C was not decreased as it was in the test for bioavailable lysine in feeding trial 2 (Table 4).

With standard procedures such as PER or NPR, our results (Table 2) agree with those of Henry et al. (13), who reported that NDM containing less than 5% moisture could be stored at 28.5 C for 2 yr without detectable loss in nutritive value of protein. In addition, White et al. (26) showed that dry whole milk of 1.9% moisture content could be stored for at least 6 mo at 37 C in tin cans without loss of biological value or true digestibility of the proteins. Erbersdobler and Zucker (9) reported only slight decreases in available lysine in spray dried skim milk powders with increasing storage time; some samples had been stored for 7 yr.

Our results show no measurable changes in protein quality of stored NDM by biological evaluation unless diets fed were adjusted to make lysine the limiting amino acid (Table 4). With lysine-limiting diets, Zimmermann (27) reported a 25% decrease in PER and a 16% decrease in NPR for vacuum packed NDM containing 3.4% moisture and stored at 40 C for 1 yr. We agree with Zimmermann that decreases in lysine bioavailability of NDM during storage at elevated temperatures could be detected provided lysine-limiting diets were fed, but the decreases we measured were much less. Zimmermann (27) and Ben-Gera and Zimmermann (4) reported decreases in chemically measured available lysine ranging from 16 to 18% in samples stored at 20, 30, and 40 C for 1 yr; the decrease in total lysine in their samples averaged 10%. Our control sample (-18 C) showed no appreciable decrease either in total lysine or in available lysine over the storage period. We found decreases in available lysine only in samples stored at 37 C; no significant decrease in total lysine was observed.

The reference (3) cited by Kramer as the source of his information regarding the nutritional stability of NDM during storage was unavailable to us. However, in (28) from the same source, all biological evaluations were with lysine-limiting diets; no data were given for results from feeding standard diets. We concluded, therefore, that Kramer based his statements about the nutritional storage stability of NDM on data from feeding trials with lysine-limiting diets. By using specific methods for lysine availability, we also found apparent changes under some storage conditions. However, because lysine is not the limiting amino acid in NDM (20) and because our results

showed no marked decline in PER and NPR even during extended storage at 37 C, Kramer's conclusions on the necessity for refrigerated and frozen storage of adequately packaged NDM are not confirmed.

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