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## TECHNICAL INFORMATION

Catalog Number: 905452, 905453, 960096, 960097, 960353, 960354, 960355, 960295, 960296, 960356, 960357, 960358  
**American Institute of Nutrition AIN-76 Semipurified Diet**

Recommendations of Ad Hoc Committee on Standards for Nutritional Studies

20 March 1980

The Committee has communicated with a number of investigators who have been using the AIN-76 diet since its first report in 1977 (*J. Nutr.*, v. 1, 1340, 1977). In studies with both rats and mice these investigators generally have had very good results in terms of animal growth, appearance and survival. A few reports have come to the Committee's attention, however, of problems encountered when the diet was modified or possibly stored improperly. The primary problem appeared to be excessive destruction of menadione or interference with its utilization, with a resultant Vitamin K deficiency, and has been noted previously in the *Journal of Nutrition*, vol. 109, p. 924, 1979.

A case of marked rancidity when the diet was not kept refrigerated has also been noted.

Two laboratories which fed the AIN-76 diet to mice for a year or longer reported a significant number of trichobezoars (hair balls) upon autopsy. Without controls fed other purified diets, the relevance of these observations to this diet is unknown. There have been comments from AIN members questioning the use of sucrose as the predominant carbohydrate. The possible adverse effect of sucrose, particularly in long term studies, were mentioned in the original report. Alternatives which have been suggested are (1) substituting glucose for sucrose, (2) replacing at least half of the sucrose with glucose, (3) using all corn starch and no sugars, (4) variable proportions of glucose, sucrose and starch depending on the nature of the study in question. It should be pointed out that commercial glucose (dextrose) is hydrated; anhydrous glucose is more expensive.

In view of the above observations, the Committee makes the following recommendations regarding the AIN-76 diet:

- (1) The amount of Vitamin K source be increased 10-fold to 500 ug/kg diet, and that a stabilized form of menadione be used, such as menadione sodium bisulfate complex (MSBC) or menadione dimethylpyrimidinol bisulfite (MPB). When thus modified, the diet should be designated AIN-76A.
- (2) That an antioxidant be added to the corn oil (or other oil if substituted), as noted in table 2, footnote 4 of the original report (*J. Nutr.*, v. 107: 1340, 1977). Satisfactory are BHT or Santoquin at 0.01-0.02% of oil. Investigators should be aware that the most popular brand of corn oil has no added antioxidant. The endogenous tocopherols are not very effective in stabilizing the oil when it is mixed into purified diets.
- (3) That investigators modify the sucrose content as noted above, if they believe the metabolic effects of sucrose may complicate their experimental plan.

Ad Hoc Committee on Standards for Nutritional Studies John G. Bieri, Chairman

### References:

- Bieri, J.G., Stoewsand, G.S., Briggs, G.M., Phillips, R.W., Woodard, J.C., and Knapka, J.J., "Report of the American Institution of Nutrition Ad Hoc Committee on Standards for Nutritional Studies," *J. Nutr.*, v. 107: 1340-1348 (1977).
- Bieri, J.G., "Second Report of the Ad Hoc Committee on Standards for Nutritional Studies," *J. Nutr.*, v. 110: 1726 (1980).

The AIN-76 diet typically provides 3.86 kcal/gm diet (3.6 kcal digestible). Total diet contains approximately 1270 ppm Sodium (0.13%), ~5500 ppm calcium (0.55%), ~5600 ppm phosphorus (0.56%), ~5400 ppm potassium, and 0.04% Iron.

### Composition of the AIN-76 Semipurified Diet:

Ingredient	Amount
Casein Purified High Nitrogen	20.0%

DL-Methionine	0.3%
Sucrose	50.0%
Corn Starch	15.0%
Alphacel, Non-Nutritive Bulk	5.0%
Corn Oil	5.0%
Choline Bitartrate	0.2%
AIN Mineral Mix (below in grams/kg of Mineral Mix)	3.5%
Calcium Phosphate Dibasic	500.00 gm
Sodium Chloride	74.00 gm
Potassium Citrate Monohydrate	220.00 gm
Potassium Sulfate	52.00 gm
Magnesium Oxide	24.00 gm
Manganese Carbonate (43-48% Mn)	3.50 gm
Ferric Citrate (16-17% Fe)	6.00 gm
Zinc Carbonate (70% ZnO)	1.60 gm
Cupric Carbonate (53-55% Cu)	0.30 gm
Potassium Iodate	0.01 gm
Sodium Selenite	0.01 gm
Chromium Potassium Sulfate	0.55 gm
Sucrose, finely powdered	118.00 gm
AIN Vitamin Mix (below are per kg of Vitamin Mix)	1.0%
Thiamine Hydrochloride	0.6 gm
Riboflavin	0.6 gm

Pyridoxine Hydrochloride	0.7 gm
Nicotinic Acid	3.0 gm
D-Calcium Pantothenate	1.6 gm
Folic Acid	0.2 gm
D-Biotin	0.02 gm
Cyanocobalamin (Vitamin B <sub>12</sub> )	0.001 gm
Retinyl Palmitate (Vitamin A) Pre-mix (250,000 IU/gm)	1.6 gm
DL-a-Tocopherol Acetate (250 IU/gm)	20.0 gm
Cholecalciferol (Vitamin D <sub>3</sub> , 400,000 IU/gm)	0.25 gm
Menaquinone (Vitamin K <sub>2</sub> )	0.005 gm
Sucrose, finely powdered	972.9 gm

The exact formulation for this diet recommended by the American Institute of Nutrition is intended for growth and maintenance during the first year of life. Investigators should be aware that diets high in sucrose can cause hepatic steatosis, can be cariogenic, and that some strains of rats fed such diets may develop kidney lesions after extended periods. The diet has been found to be satisfactory for reproduction and lactation in both rats and mice. If used for deficiency studies, modifications will be necessary. If used in ultra-clean environment, several trace elements should be added (*Fed. Proc.*, v. **33**, 1748, 1767, 1773, 1974). **Note:** See Second Report of the Ad Hoc Committee on Standards for Nutritional Studies.

**Availability:**

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
905452	Powdered	10 kg
		20 kg
		50 kg
905453	Pelleted	10 kg
		20 kg
		50 kg

**Also Available:**

**AIN-76A Semipurified Diet**

The amount of Vitamin K source is increased 10-fold to 500 ug/kg diet and a stabilized form of menadione is used, such as menadione sodium bisulfite.

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
960096	Powdered	10 kg 20 kg 50 kg
960097	Pelleted	10 kg 20 kg 50 kg

#### **AIN-76 B-40**

AIN-76A diet with 40% corn starch and 25% sucrose.

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
960353	Powdered	10 kg 20 kg 50 kg
960354	Pelleted	10 kg 20 kg 50 kg

#### **AIN-76 B-65**

AIN-76A diet with 65% corn starch (Not available in Pellet Form).

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
960355	Powdered	10 kg 20 kg 50 kg

#### **AIN-76C Semipurified Diet**

An antioxidant is added to the corn oil. Investigators should be aware that the most popular brands of corn oil have no added antioxidant and that the endogenous tocopherols are not very effective in stabilizing the oil when it is mixed into purified diets. This diet contains 500 ug/kg menadione sodium bisulfite, BHT (Butylated Hydroxy Toluene), 0.02% per gram of oil.

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
960295	Powdered	10 kg 20 kg 50 kg
960296	Pelleted	10 kg 20 kg 50 kg

#### **AIN-76 D-40**

AIN-76C diet with 40% corn starch and 25% sucrose.

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
960356	Powdered	10 kg 20 kg 50 kg
960357	Pelleted	10 kg 20 kg 50 kg

#### **AIN-76 D-65**

AIN-76C diet with 65% corn starch (Not available in Pellet Form).

<i>Catalog Number</i>	<i>Description</i>	<i>Size</i>
960358	Powdered	10 kg 20 kg 50 kg

