

Alcohol Consumption and Vehicle Preference in Young Sinclair(S-1) Miniature Swine Fed Two Levels of Dietary Protein (36828)

A. M. PRESTON, M. E. TUMBLESON, D. P. HUTCHESON, AND C. C. MIDDLETON

Sinclair Comparative Medicine Research Farm and Department of Veterinary Physiology and Pharmacology, University of Missouri, Columbia, Missouri 65201

Chronic alcoholism in humans is characterized by a low protein, high carbohydrate intake with alcohol frequently accounting for 40% or more of total ingested calories (1, 2). Voluntary consumption of alcohol at this level has not yet been duplicated in laboratory animals. The most commonly used animal, the rodent, will consume ethanol to provide only 25 to 30% of the total calories even when ethanol is the only available fluid (3). Incorporation of ethanol into alcohol-liquid diets of mice has permitted 45 to 60% derived calories as ethanol (4), but this method precludes the natural selection of alcohol vs food, which is unlike the human conditions. Identification of an animal with a greater preference for alcohol is therefore desirable.

Due to similarities in anatomy, physiology and size of man, miniature swine are frequently used in studies related to human health (5, 6). Recently, Hutcheson, Brown and Middleton (7) have shown that the amount of alcohol consumed by adult miniature swine is affected by protein and caloric content of the diet. The purposes of this study were to investigate the quantity of alcohol consumed by young miniature swine as a function of dietary protein and caloric content of the diet, to determine the influence of vehicle on intake of ethanol and to assess the suitability of our strain of miniature swine as a biomedical model for alcohol studies.

Materials and Methods. Thirty-two Sinclair(S-1) miniature pigs, 10 wk of age, were used in a $2 \times 2 \times 2$ factorial arrangement of treatments involving percentage protein of the diet, level of feed and access to alcohol. Animals were housed as described previously (8) and were limit fed 16 or 8%

protein diets (Table I). The intake levels were calculated weekly on the basis of twice maintenance feed levels for the upper feed intake group and one half this amount for the lower feed intake group. The diets were isocalorically formulated and contained NRC requirements for minerals and vitamins. Diets were fed in pellet form for the first 6 wk on trial and in meal form the remaining 9 wk. Antibiotic was added to the meal form at the level shown in Table I.

Experimental pigs had free choice of water or ethanol solutions (w/v) prepared from 95% laboratory alcohol. The first 3 wk on trial (period 1), the pigs had a choice of water, 10% ethanol in water, 10% ethanol in cola or 10% ethanol in orange juice. The second 3 wk (period 2), the pigs had a choice of water, 20% ethanol in water, 20% ethanol in cola or 20% ethanol in orange juice. The third 3 wk (period 3), the pigs had a choice of water, 10% ethanol in water or 20% ethanol in water. The fourth 3 wk (period 4), the pigs had a choice of water or 30% ethanol in orange juice. The fifth 3 wk (period 5), the pigs had a choice of water or 40% ethanol in orange juice. Control pigs were given water *ad libitum*. During periods 4 and 5, control pigs were allowed water and free choice orange juice up to the limit of that consumed by experimental pigs. Liquid intakes were recorded 3 times/wk. Correction was made for evaporation of ethanol, which was approximately 10%/day in each solution. Consumption results were converted to 100% ethanol consumed on a percentage by weight basis.

Body weights were recorded weekly and feed levels were formulated. Biweekly blood samples were collected by jugular venipuncture and plasma ethanol was determined via

TABLE I. Composition of Diets.

Ingredients (kg/100 kg diet)	Protein (%) in diet	
	16	8
Corn, grain, 9% protein	72.6	36.3
Cornstarch	—	46.2
Soybean meal, 44% protein	15.2	7.6
Alfalfa meal, 17% protein	2.4	1.2
Meat meal, 51% protein	5.0	2.5
Dicalcium phosphate	0.6	2.2
Ground limestone	0.5	0.3
Salt	0.5	0.5
Vitamin-antibiotic mix ^a	1.0	1.0
Binder	2.2	2.2
Digestible energy (kcal/kg)	3300	3310

^a Provided the following per kg of complete diet: vitamin A, 2640 IU; vitamin D, 704 IU; riboflavin, 70.4 mg; pantothenic acid, 14.1 mg; nicotinic acid, 14.1 mg; vitamin B₁₂, 35.2 µg; zinc, 88 mg; and a 2:2:1 mixture of chlortetracycline:sulfamethiazine:penicillin, 220 mg.

the enzymatic method (Sigma Chemical Co.). Additional blood samples were collected from animals fed the 16% protein diet on week 13 and from all animals on week 15, after which the animals were necropsied (8).

The data were subjected to analysis of variance techniques with all hypotheses tested at $p < .01$.

Results. The mean (\pm SE) initial weight of all animals was 5.47 ± 0.28 kg. Control pigs fed 16% protein diet at the upper level ($N = 4$) weighed significantly more during periods 2 through 5 than all other groups of animals (Table II). Experimental pigs fed the 16% protein diet at the upper level ($N = 4$) weighed significantly more during period 5 than did animals fed the 8% protein diet ($N = 14$). Control pigs fed the 8% protein diet at the upper level ($N = 2$) weighed significantly less, during periods 2 through 5, than any other group of animals; two animals in this group stopped eating and died of apparent starvation. Animals fed the 16% protein diet at the upper level ($N = 4$) drank significantly more water and total fluid in periods 1, 2, 4 and 5 than any other group of animals (Table II). Experimental pigs fed the 16% diet ($N = 8$) drank signifi-

cantly more water and total fluid during period 5 than any other such group of animals. Total fluid intake increased markedly for control animals ($N = 14$) in periods 4 and 5 when orange juice was made available. Total calories consumed followed percentage protein and intake of the diet with animals fed the 16% protein diet at the upper level ($N = 4$) consuming the most calories and animals fed the 8% protein diet at the lower level ($N = 4$) consuming the least calories (Table II). There were no differences in total calories consumed between control and experimental animals within similar treatments.

Taste preference for ethanol in water, cola or orange juice was determined during the first two periods. Animals fed the 16% protein diet ($N = 8$) drank ethanol in orange juice in a significantly greater amount than in cola or water during period 1; animals fed 16% ($N = 8$) or 8% ($N = 8$) protein diets drank ethanol in orange juice in significantly greater amounts than in cola or water during period 2. Orange juice was, therefore, selected as the preferred carrier for ethanol for periods 4 and 5.

Alcohol consumption increased during peri-

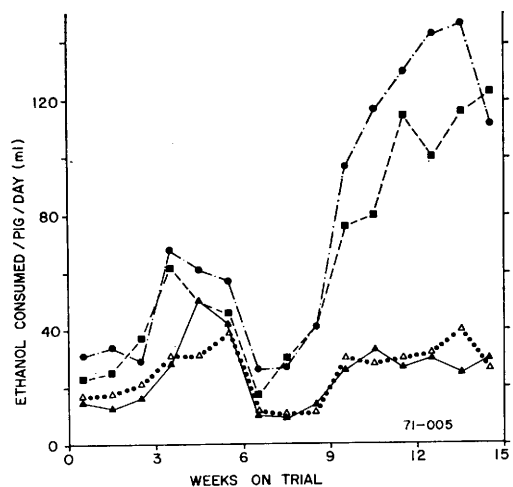


FIG. 1. Amount of 100% ethanol consumed/pig/day by animals fed: (■- -) 16% protein diet at the upper feed level; (●- ·) 16% protein diet at the lower feed level; (△...) 8% protein diet at the upper feed level; (▲—) 8% protein diet at the lower feed level. Each value represents the mean of 4 pigs.

TABLE II. Body Weight, Fluid, Feed and Caloric Intake Data of Animals Fed 16 or 8% Protein Diets at the Upper Feed Level or at the Lower Feed Level, Consuming Water and Ethanol Solutions (Experimental Pigs) or Water Alone (Control Pigs).

Treatment: Period:	16% Protein										8% Protein									
	Upper feed level					Lower feed level					Upper feed level					Lower feed level				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Mean body wt (kg)																				
Experimental	6.59	7.86	9.58	12.41	16.21	6.19	7.35	8.23	9.82	11.80	6.55	7.67	9.02	10.88	12.80	5.51	5.61	5.90	6.46	8.26
Control	6.62	8.76	12.81	17.00	21.51	6.11	7.23	8.75	10.54	13.07	5.02	4.61	4.68	4.76	5.59	5.03	5.59	6.22	6.96	8.16
Water consumed/pig/ day (liters)																				
Experimental	0.39	0.52	0.71	1.30	2.03	0.22	0.34	0.55	0.76	1.00	0.22	0.30	0.55	0.67	0.85	0.19	0.21	0.39	0.47	0.60
Control	0.41	0.77	0.84	1.03	1.31	0.30	0.38	0.58	0.53	0.73	0.20	0.18	0.25	0.38	0.64	0.19	0.27	0.36	0.35	0.54
Fluid consumed/pig/ day (liters)																				
Experimental	0.67	0.78	0.93	1.60	2.31	0.54	0.65	0.75	1.13	1.34	0.40	0.46	0.61	0.77	0.93	0.34	0.41	0.46	0.57	0.67
Control	0.41	0.77	0.84	1.53	1.83	0.30	0.38	0.58	1.09	1.22	0.20	0.18	0.25	0.60	0.81	0.19	0.27	0.36	0.62	0.76
Feed consumed/pig/ day (kg)																				
Experimental	0.30	0.32	0.43	0.52	0.65	0.16	0.19	0.22	0.26	0.33	0.34	0.37	0.43	0.48	0.55	0.17	0.19	0.21	0.23	0.28
Control	0.31	0.40	0.48	0.58	0.75	0.16	0.20	0.25	0.29	0.38	0.27	0.23	0.26	0.38	0.54	0.15	0.19	0.22	0.24	0.28
Energy consumed/pig/ day (kcal)																				
Experimental	1271	1449	1592	2326	2858	855	1095	889	1628	1919	1303	1489	1472	1771	2022	713	920	749	972	1090
Control	1009	1320	1623	2116	2732	535	661	812	1196	1468	886	774	852	1363	1849	491	627	713	908	1007

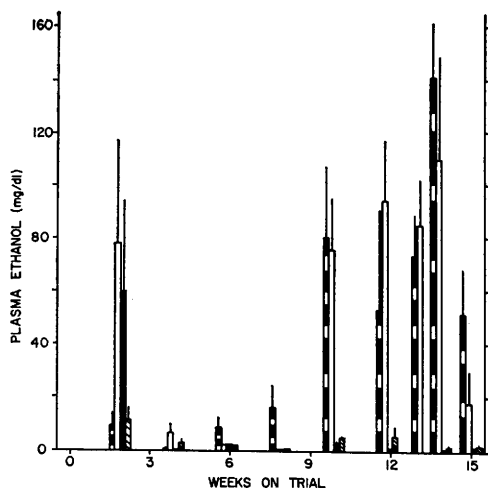


FIG. 2. Plasma ethanol in animals fed: (striped bars) 16% protein diet at the upper feed level; (white bars) 16% protein diet at the lower feed level; (black bars) 8% protein diet at the upper feed level; (diagonal lined bars) 8% protein diet at the lower feed level. Each value represents the mean + SE of 4 pigs.

ods 1 and 2, decreased during period 3 when water was the sole carrier, but increased markedly in periods 4 and 5 when orange juice was the ethanol carrier (Fig. 1). Plasma ethanol (Fig. 2) and percentage of calories (Fig. 3) derived from ethanol data follow the consumption pattern. Plasma ethanol concentrations from control animals were always less than 3 mg/dl and initial plasma ethanol concentrations were less than 1 mg/dl in all animals.

Physical signs of intoxication were noted in period 1 in animals consuming alcohol; however, during the remainder of the study, even in periods 4 and 5 when high ethanol intake occurred, the animals showed no physical manifestations of intoxication.

Discussion. Weight changes of animals fed 16 or 8% protein diets were similar to those reported by Tumbleson *et al.* (9) for young miniature swine. Ethanol consumption was greater consistently for animals fed the 16% protein diet than for those fed the 8% protein diet. The animals fed the 16% protein diet may have required additional calories to metabolize the greater amount of protein. The percentage of calories derived from ethanol

was greater for animals fed the 16% protein diet than those fed 8% protein diet. Animals fed at the lower feed level derived a significantly greater percentage of their calories from ethanol than animals fed at the upper feed level; however, this result was undoubtedly affected by the restricted caloric intake.

Tolerance to ethanol is a well-documented characteristic of human alcoholics (10, 11) and in rodents (12, 13). As indicated by plasma ethanol values, pigs fed the 16% protein diet could not metabolize all ingested alcohol during periods 4 and 5. Absence of physical signs of intoxication might indicate that these pigs had developed a tolerance for alcohol at the levels consumed.

The damaging effects of alcohol in humans results when food intake is reduced and proteins and vitamins intake are below required levels. In this study, pigs did not decrease their food intake; however, 40% of the total ingested calories were in the form of ethanol in animals fed the 16% protein diet at the lower feed level. When rodents are fed in excess of 30% of total calories as ethanol, conventionally adequate diets cannot prevent serious imbalances from resulting in the to-

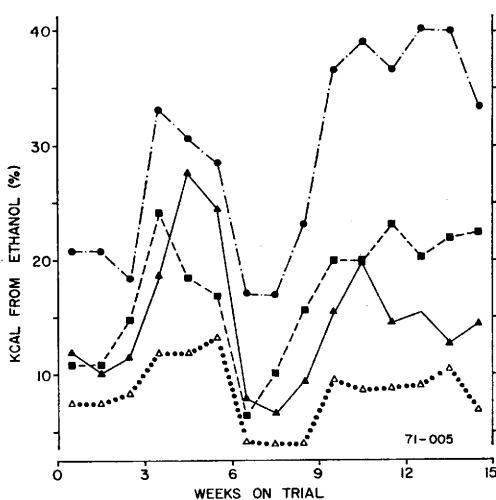


FIG. 3. Percentage of calories derived from ethanol in animals fed: (■- -) 16% protein diet at the upper feed level; (●- -) 16% protein diet at the lower feed level; (△..) 8% protein diet at the upper feed level; (▲—) 8% protein diet at the lower feed level. Each value represents the mean of 4 pigs.

tal dietary regimen (3). Whether or not such a condition was reached in this study is not known. However, the important criteria of developing a laboratory animal with preference for alcohol and one that will consume a high percentage of calories in the form of ethanol has been demonstrated with young miniature swine.

Summary. The quantity of alcohol consumed by young miniature swine was determined as a function of dietary protein and caloric intake. Pigs were fed 16% or 8% protein diets at two levels, the upper level being twice the lower level. Animals eating the 16% protein diet consumed more alcohol than those animals fed the 8% protein diet. Animals fed the lower food levels obtained a greater percentage of calories from alcohol than animals fed the upper food levels. Pigs fed the 16% protein diet at the lower level obtained 40% of total calories as ethanol. Orange juice was preferred by pigs as a carrier of ethanol over cola or water.

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Received June 5, 1972. P.S.E.B.M., 1972, Vol. 141.