

Dietary Induction of Mulberry Heart Disease and Hepatosis Dietetica in Pigs I. Nutritional Aspects

B. A. Sharp, L. G. Young and A. A. van Dreumel*

ABSTRACT

Two trials were conducted, involving 48 Yorkshire specific pathogen-free pigs, three to four weeks old, to investigate diets which would result in a high incidence of deaths from mulberry heart disease and hepatosis dietetica in pigs. Diets based on ground corn with torula yeast resulted in a high incidence of death. Protein supplements of dried skim milk or soybean meal with corn did not induce a high incidence of death. Diets supplemented with torula yeast had the lowest selenium concentration and highest α -tocopherol concentration of the diets investigated and resulted in lower liver selenium concentrations. A higher frequency of hepatosis dietetica, mulberry heart disease, skeletal muscle degeneration and exudative diathesis was observed in pigs fed the low level selenium diets containing torula yeast.

RÉSUMÉ

Les auteurs ont effectué deux expériences, en utilisant 48 porcelets gnotobiotiques Yorkshire âgés de trois à quatre semaines, dans le but d'étudier des diètes qui engendreraient plusieurs cas mortels de cardiopathie mûriforme et d'hépatose diététique porcines. Des diètes à base de maïs moulu et enrichies de levure torula causèrent plusieurs mortalités. Des suppléments protéiques: lait écrémé en poudre et tourteau de soya, ajoutés à du maïs, ne causèrent que quelques mortalités. Les diètes enrichies de levure torula contenaient la plus fai-

ble concentration en sélénium et la plus forte en α -tocophérol de toutes les diètes expérimentales et produisirent de faibles concentrations hépatiques en sélénium. L'hépatose diététique, la cardiopathie mûriforme, la dégénérescence des muscles squelettiques et la diathèse exsudative s'avèrent plus fréquentes chez les porcelets nourris avec les rations à faible teneur en sélénium qui contenaient de la levure torula.

INTRODUCTION

During recent years in Ontario there has been an apparent increase in sudden deaths in swine exhibiting lesions similar to those described by Obel (21) for hepatosis dietetica (HD) and by Grant (11) for mulberry heart disease (MHD). Both workers reported a high incidence of skeletal muscle degeneration (SMD), exudative diathesis (ED) and microangiopathy (MAP) in association with HD and MHD. Obel (21) also observed gastric ulcers in many pigs with HD. Low levels of vitamin E (21) and selenium (11) have been implicated in the conditions. It has been suggested that practical swine diets would not likely be deficient in vitamin E (20). The routine supplementation of swine diets with selenium has not been allowed by regulatory authorities. Most of the earlier work regarding these conditions was conducted in Sweden; however, the feedstuffs used and conditions under which swine are raised differ from that in Canada.

Therefore an investigation into the problem as observed in Canada appeared warranted. The objective of this work was to develop diets which would produce a high frequency of MHD and HD in pigs. Torula yeast was used as the main protein supplement since it is low in Se (4, 19, 22, 24).

*Department of Animal Science (Sharp and Young) and Department of Pathology (van Dreumel), University of Guelph, Guelph, Ontario.

Present address of B. A. Sharp: Canadian Simmental Association, 108A-8th Avenue, S.W., Calgary 2, Alberta. A. A. van Dreumel: Veterinary Services Laboratory, Veterinary Services Branch, Ontario Department of Agriculture and Food, Guelph, Ontario.

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GENERAL

A total of 48 Yorkshire specific pathogen-free (SPF) pigs, from sows which had not been supplemented with selenium or vitamin E, were used in two trials. The pigs had access to a similar diet prior to initiation of the trial. The pigs were confined to metal pens with a woven wire floor elevated about 0.30 m above a concrete floor in an enclosed heated building. Feed and water were given *ad libitum* via self feeders and water nipples.

All pigs which died during, or were killed at the termination of the trials, were necropsied within 12 hours after death. Surviving pigs were killed by electrocution. Samples of liver, muscle, kidney, heart and spleen were obtained and stored in separate plastic bags at -20°C for later chemical analyses. Sections of heart, liver, kidney, brain, adrenals, spleen, skeletal muscle, skin and bone marrow, were fixed and retained in 10% formalin for histological examination.

FEED AND DIET PREPARATION

TRIAL 1

The corn used in the two trials was harvested in the fall of 1967 at the Elora Research Station and artificially dried to approximately 85% dry matter before storage. The dried skim milk¹ was of one batch, processed during February 1967. The torula yeast² was purchased in one lot. The soybean meal used in the second trial was imported from Ohio. The corn was ground to a medium to fine consistency with a hammermill prior to diet preparation. The diets were mixed weekly or when needed in a 100 kg capacity mixer and stored in plastic bags.

Thirty pigs averaging 6.3 kg and approximately 24 days of age from five litters were assigned to individual pens such that a littermate pair, an intact male and female, received one of three diets in a 2 x 3 (sex and diets) factorial randomized complete block arrangement. Diets calculated to contain 20% crude protein were compounded using corn and either torula yeast or dried skim milk as the additional source of protein (Table I). The heated corn used in diet 1215 was prepared by heating the ground corn in a forced draft at 100°C for 24 hours. This was done in an attempt to reduce the level of α -tocopherol in the diet. The duration of the trial was 140 days.

¹Gay Lea Dairy, Tara, Ontario.
²Lakes States Yeast Corp., Rhinelander, Wis.

TABLE I. Composition of Experimental Diets^a

Diet No.	Trial 1			Trial 2		
	1214	1215	1216	1221 ^b	1222 ^b	1223
Ingredient	Percent					
Corn	76.00		57.25	76.00	57.27	70.40
Heated corn ^c		57.25				
Torula yeast	20.90			20.90		
Dried skim milk		40.90	40.90		40.90	
Soybean meal						26.00
Vitamin premix 1003 ^a	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix 1004 ^a	0.10	0.10	0.10	0.10	0.10	0.10
Cobalt iodized salt	0.50	0.50	0.50	0.50	0.50	0.50
Calcium phosphate (18.5% Ca, 20% P)	0.50	0.25	0.25	0.50	0.25	1.30
Limestone	1.50	0.50	0.50	1.50	0.50	1.20

^aTrace mineral premix supplied the following ppm in the complete diet: manganese 60, iron 70, copper 10, zinc 100
 Vitamin premix supplied the following per kg of diet: riboflavin 4.4 mg, pantothenic acid 8.8 mg, niacin 19.9 mg, choline chloride 110.3 mg, vitamin B₁₂ 19.9 ug, vitamin A 3,309 IU, vitamin D₂ 330.9 IU.
^bComplete diets heated at 70°C for 24 hours in a forced draft oven
^cGround corn heated at 100°C for 24 hours in a forced draft oven

TRIAL 2

Eighteen pigs averaging 6.9 kg and approximately 25 days of age from three litters were allotted to three treatments such that a littermate pair, a male and female, were penned together and fed one of the diets (Table I) in a 2 x 3 factorial (sex and diets) randomized complete block arrangement. The heated torula yeast-corn and heated dried skim milk-corn diets were

the same formulae as used in the first trial, except the complete diets were heated at 70°C for 24 hours in a forced draft oven.

The pigs were maintained on trial until death or for 70 days at which time the survivors were electrocuted and necropsied.

SELENIUM ASSAY

The fluorometric procedure of Hoffman *et al* (14) was used for selenium assay. The

TABLE II. Pig Performance, Dietary and Tissue Vitamin E and Selenium Concentrations

Diet Number	Trial 1			Trial 2		
	1214	1215	1216	1221	1222	1223
Av. daily feed kg dry matter ^a	0.82 ¹	0.87 ¹	1.20 ²	0.60 ¹	0.93 ¹	0.96 ¹
Av. daily gain kg ^a	0.26 ¹	0.46 ²	0.49 ²	0.22 ¹	0.49 ²	0.43 ²
Av. gain/feed ^a	0.36 ¹	0.59 ³	0.49 ²	0.38 ¹	0.53 ²	0.46 ^{1,2}
Died/total	9/10	1/10	1/10	4/6	1/6	1/6
Mean days of survival	59.8 ¹	131.9 ²	124.8 ²	57.8 ¹	64.7 ¹	62.7 ¹
Feed selenium ug/g ^b	0.021	0.067	0.072	0.034	0.055	0.043
Feed tocopherol ug/g ^b	16.50	2.16	2.58	15.92	2.70	4.46
Liver selenium ug/g ^b	0.171 ¹	1.299 ²	1.238 ²	0.216 ¹	0.605 ²	0.286 ¹

^aFor the first seven weeks of the trial

^bDry matter basis

^{1,2,3}—means on the same line for each trial bearing different superscripts differ significantly (P = 0.05)

TABLE III. Incidence of Lesions and Death of Pigs Fed the Experimental Diets, Trials 1 and 2

Source	Died/Total	Hepatitis Dietetica	Myocardial Degeneration	Skeletal Muscle Degeneration	Exudative Diathesis	Gastric Lesions*
Trial 1						
Replication 1	1/6	1	1	3	1	3
2	2/6	2	2	4	2	5
3	2/6	1	2	2	2	5
4	2/6	2	2	2	2	4
5	4/6	3	3	3	2	2
Sex Female	6/15	5	5	9	4	10
Male	5/15	4	5	5	5	9
Diet 1214	9/10	8	9	10	9	8
1215	1/10	1	1	2	0	6
1216	1/10	0	0	2	0	5
Trial 2						
Replication 1	2/6	1	3	3	2	2
2	3/6	2	3	3	2	2
3	1/6	0	0	0	0	2
Sex Female	3/9	1	3	3	1	2
Male	3/9	2	3	3	3	4
Diet 1221	4/6	3	4	4	3	4
1222	1/6	0	1	1	0	1
1223	1/6	0	1	1	1	1

*Erosions and/or ulceration of the pars esophagea

frozen tissue was sampled, weighed and transferred to the digestion flask in a cold laboratory to minimize selenium losses.

FEED TOCOPHEROL ANALYSIS

The feeds were extracted and saponified according to the procedure proposed by the Analytical Methods Committee (3). Total tocopherols were eluted from the nonsaponifiables using a Florisil column (6) and were separated on a silica gel G thin layer plate using the solvent system described by Herting and Drury (13). Tocopherols were visualized using 2'7'-dichlorofluorescein and short wave violet light. The colorimetric procedure of Tsen (27) was used to estimate α -tocopherol content. An internal standard was added to one of a duplicate set of samples and the recovery was used to correct for tocopherol losses during the assay procedure. Values reported are α -tocopherol.

STATISTICAL ANALYSIS

The data were evaluated by the analysis of variance technique and treatment means were compared by the least significant difference method (25).

RESULTS

DEATHS AND SURVIVAL TIMES

One pig fed each of the diets containing dried skim milk or soybean meal died, while nine out of ten, and four out of six, fed torula yeast died in Trials 1 and 2 respectively. It was observed in Trial 1 that the pigs fed the torula yeast diet survived an average of about 60 days; therefore, the second trial was concluded after 70 days. The differences in survival time in Trial 2 were not significant.

FREQUENCY OF LESIONS

Detailed descriptions of gross and microscopic lesions observed in these trials are to be published in this journal at a later date. The observations made comparing the frequencies of the various lesions

were not analyzed statistically since only a subjective evaluation of the severity of lesions was made. It would appear that neither replication (litter) nor sex had any effect on the incidence of HD or MHD in Trial 1 (Table III). Female pigs were more frequently affected with SMD than male pigs. There was a considerably greater frequency of the various lesions, with the possible exception of gastric lesions, in pigs fed the torula yeast diet. All ten of the pigs fed the torula yeast diet exhibited SMD, nine exhibited MHD and ED, and eight exhibited HD and erosions and/or ulceration of pars esophagea. Fifty percent or more of the pigs fed each of the diets exhibited gastric lesions (erosions and/or ulceration).

Sex of the pig did not appear to influence the development of the lesions in Trial 2. Pigs fed the torula yeast diets exhibited a greater frequency of lesions and death as was observed in the first trial.

Heating of the diets did not have a marked influence on pig survival or frequency of lesions.

DIETARY TOCOPHEROL AND SELENIUM AND TISSUE SELENIUM CONCENTRATIONS

The dried skim milk diets had between two and three times as much selenium as the torula yeast diets (Table II). In Trial 2, the soybean meal diet contained an intermediate level of selenium between the other two diets. The torula yeast diet had much higher tocopherol values than the other diets.

Pigs fed the dried skim milk diets had the highest liver selenium levels. In Trial 1 the liver selenium levels of pigs fed the dried skim milk diets were approximately 7.5 times higher (1.299 and 1.238 $\mu\text{g/g}$) than those of pigs fed torula yeast (0.171 $\mu\text{g/g}$); however, the corresponding dietary selenium levels were about three times greater in the dried skim milk diets. These differences were of a lower magnitude in Trial 2.

GROWTH AND FEED CONSUMPTION

Although the differences were not statistically significant, the pigs fed the torula yeast diets tended to gain the slowest, ate less feed per day and had lower gain/feed ratios.

DISCUSSION

The survival time and the frequency of death of pigs fed diets containing torula yeast are similar between trials and correspond with other reports (7, 9, 11, 21).

Various researchers have used torula yeast to induce HD in swine (7, 9, 19, 22). The reasons that torula yeast readily produces the necrosis observed in our work and others are: a) it is low in selenium (0.009 ug/g) (8); b) it is high in unsaturated fatty acids (4, 16) and c) the yeast may contain a selenium or tocopherol antagonist (23, 24). The torula yeast in these trials contained 0.018 ug of selenium per gram of dry yeast. The level of feed tocopherol appeared to be inversely related to the incidence of lesions and death. Thus the lesions observed may have been due to an antagonist of vitamin E and/or selenium or a deficiency of selenium or a combination of both.

Low tocopherol levels (0.037 mg/g) (2) and relatively low selenium levels (0.075 to 0.15 ppm) (26) would indicate that dried skim milk would serve as an excellent protein supplement to produce lesions. The dried skim milk used in these trials contained 0.11 ppm selenium and the data presented does not support the use of this protein supplement for the induction of vitamin E-selenium deficiency lesions.

Protein derived from soybeans has been used to induce lesions, but usually in conjunction with unsaturated oils (9, 11, 15). The corn-soybean meal diet used in Trial 2 failed to induce sufficient numbers or severity of lesions to facilitate the study of the vitamin E-selenium deficiency syndrome. Groce *et al* (12) have observed a similar low incidence of lesions when pigs were fed a corn-soybean meal diet which contained approximately the same levels of selenium and α -tocopherol as the diet used in Trial 2.

The lower liver selenium level of pigs fed diets containing dried skim milk in Trial 2 as compared with Trial 1 may be partially explained by the lower dietary selenium concentration in Trial 2. The difference in the duration of the two trials (140 vs 70 days) may also have a bearing on the liver selenium levels observed.

A liver level of 0.2 ppm selenium (dry basis) has been suggested by Mathias *et al* (18) for rats, Cousins and Cairney (5) and Allaway *et al* (1) for sheep and Lindberg and Siren (17) for pigs as the mini-

mal concentration which will protect the animals from selenium responsive diseases when fed diets low in vitamin E. This liver level corresponds well with our results and it would appear protection is afforded between 0.2 and 0.3 ppm liver selenium as demonstrated by the liver level found in the pigs fed the soybean meal diet in Trial 2 but not the torula yeast diet. However, Ewan (10), has reported considerably lower Se levels when pig diets were supplemented with vitamin E, without the appearance of deficiency symptoms.

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REFERENCES

1. ALLAWAY, W. H., D. P. MOORE, J. E. OLDFIELD and O. H. MUTH. Movement of physiological levels of selenium from soils through plants to animals. *J. Nutr.* 88: 411-418. 1966.
2. AMES, S. R. Role of vitamin E (α -tocopherol) in poultry nutrition and disease. A review of recent literature. *Poult. Sci.* 35: 145-159. 1956.
3. ANALYTICAL METHODS COMMITTEE. The determination of tocopherol in oils, foods, and feeding stuffs. *Analyst*, London 84: 356-372. 1959.
4. BIERI, J. G., G. M. BRIGGS and C. J. POLLARD. Acceleration of vitamin E deficiency by torula yeast. II. Effect of torula yeast ash and lipid. *Proc. Soc. exp. Biol. Med.* 99: 262-265. 1958.
5. COUSINS, F. B. and I. M. CAIRNEY. Some aspects of selenium metabolism in sheep. *Aust. J. agric. Res.* 12: 927-943. 1961.
6. DICKS-BUSHNELL, M. W. Column chromatography in the determination of tocopherol. Florisil, silicic acid and secondary magnesium phosphate. *J. Chromat.* 27: 96-103. 1967.
7. EGGERT, R. G., E. PATTERSON, W. J. AKERS and E. L. R. STOKSTAD. The role of vitamin E and selenium in the nutrition of the pig. *J. Anim. Sci.* 16: 1037. 1957.
8. EWAN, R. C., C. A. BAUMANN and A. L. POPE. Retention of selenium by growing lambs. *J. agric. Fd Chem.* 16: 216-219. 1968.
9. EWAN, R. C., M. E. WASTELL, E. J. BICKNELL and V. C. SPEER. Performance and deficiency symptoms of young pigs fed diets low in vitamin E and selenium. *J. Anim. Sci.* 29: 912-917. 1969.
10. EWAN, R. C. Effect of vitamin E and selenium on tissue composition of young pigs. *J. Anim. Sci.* 32: 883-887. 1971.
11. GRANT, C. A. Morphological and etiological studies of dietetic microangiopathy in pigs. Mulberry heart. *Acta vet. scand.* 2: Suppl. 3. 1961.
12. GROCE, A. W., E. R. MILLER, K. K. KEAHEY, D. E. ULLREY and D. J. ELLIS. Selenium supplementation of practical diets for growing-finishing swine. *J. Anim. Sci.* 32: 905-911. 1971.
13. HERTING, D. C. and E. J. E. DRURY. Thin-layer chromatography with precoated alumina sheets. II Application to tocopherols. *J. Chromat.* 30: 502-505. 1967.
14. HOFFMAN, I., R. J. WESTERBY and M. HIDROGLOU. Precise fluorometric microdetermination of selenium in agricultural materials. *J. Ass. off. agric. Chem.* 51: 1039-1042. 1968.
15. HOVE, E. L. and H. R. SEIBOLD. Liver necrosis and altered fat composition in vitamin E deficient swine. *J. Nutr.* 56: 173-186. 1955.

16. JENSEN, L. S. Vitamin E and essential fatty acids in avian reproduction. *Fedn Proc. (Fedn Am. Soc. exp. Biol. Baltimore)* 27: 914-919. 1968.
17. LINDBERG, P. and M. SIREN. Fluorometric selenium determination in the liver of normal pigs affected with nutritional muscular dystrophy and liver dystrophy. *Acta vet. scand.* 6: 59-64. 1965.
18. MATHIAS, M. M., W. H. ALLAWAY, D. E. HOGUE, M. V. MARION and R. W. GARDNER. Value of selenium in alfalfa for the prevention of selenium deficiency in chicks and rats. *J. Nutr.* 86: 213-219. 1965.
19. MICHEL, R. L., C. K. WHITEHAIR and K. K. KEAHEY. Dietary hepatic necrosis associated with selenium-vitamin E deficiency in swine. *J. Am. vet. med. Ass.* 155: 50-59. 1969.
20. N.R.C. Nutrient Requirements of Swine. U.S. National Academy of Sciences Pub. 1599. 1968.
21. OBEL, A. L. Studies on the morphology and etiology of so-called toxic liver dystrophy (hepatosis dietetica) in swine. *Acta path. microbiol. scan. Suppl.* 94. 1953.
22. PELLIGRINI, L. A study of vitamin E deficiency in pigs fed a torula yeast diet. Thesis. Univ. of Minnesota, Minneapolis. 1958.
23. PROCTOR, J. F., D. C. MAPLEDEN, D. E. HOGUE and J. K. LOOSLI. Relation in selenium, vitamin E and other factors to muscular dystrophy in the rabbit. *Proc. Soc. exp. Biol. Med.* 108: 77-79. 1961.
24. RAHMAN, M. M., R. E. DAVIES, C. W. DEYONE and J. R. COUCH. Selenium and torula yeast in production of exudative diathesis in chicks. *Proc. Soc. exp. Biol. Med.* 105: 227-230. 1960.
25. STEEL, R. G. D. and J. H. TORRIE. *Principles and Procedures of Statistics*, New York: McGraw-Hill Book Co. 1960.
26. THOMPSON, J. M. and M. L. SCOTT. Selenium in practical chicken feeds. *Cornell Nutr. Conf.* pp. 121-125. 1968.
27. TSEN, C. C. An improved spectrophotometric method for the determination of tocopherols using 4, 7-diphenyl-, 10 phenanthroline. *Analyt. Chem.* 33: 849-851. 1961.

Book Review

PELZTIERKRANKHEITEN. *Hans-Christoph Löliger. Published by VEB Gustav Fischer Verlag, Jena. 1970. 399 pages. Price 89.00 Mark.*

This book on diseases of fur-bearing animals covers in a comprehensive way the diseases of the economically most important fur-bearing species: fox, mink, chinchilla, nutria and raccoon. The diseases of rabbits are not included.

The book is divided into two parts. The first part comprises chapters on viral, rickettsial, bacterial and mycotic diseases, and a separate chapter on toxoplasmosis. The second part contains ten chapters on diseases of the different organ systems and three additional chapters including metabolic and deficiency diseases, diseases of the newborn and poisonings. Parasitic diseases are dealt with in the chapters on diseases of the organ systems. Naturally, some repetition could not be avoided. Nevertheless, the text is arranged well and the reader should have no difficulty in finding the information he wants. The description of each disease is subdivided in the conventional way: introduction, etiology, clinical symptoms, pathological findings, diagnosis, treatment, prophylaxis and

literature. The lists of references are by no means complete but sufficient as a guide for those readers who would like to pursue the subject in more depth. References are quoted up to 1968. Much emphasis is laid on the description of the gross pathological and histopathological changes which are illustrated by 130 photographs.

Some readers possibly would prefer to see a more detailed discussion of treatment. In most cases the required doses of drugs are not listed.

In the paragraphs on aleutian disease of mink, a more precise distinction between the Chediak-Higashi syndrome and aleutian disease of mink would have been desirable.

The Canadian reader will object to the legends of Figures 128 and 129: "... Guelph (U.S.A.)".

On the whole, this volume is an excellent source of information on diseases of fur-bearing animals for the veterinary student, the practising veterinarian and the veterinarian in a diagnostic laboratory. The book has one major drawback for the North American reader: it is written in German.—*H. Tabel.*