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CONTENTS

INFLUENCE OF NUTRITION AND ENVIRONMENT ON PERFORMANCE OF MARKET TURKEYS

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Studies conducted at the Northwest Experiment Station (Crookston), University of Minnesota, in the 1950s (1) showed that turkeys fed a 25% protein supplement, corn, and oats (free choice) after 8 weeks of age voluntarily reduced their percentage dietary protein intake with time, similar to practice in present day complete feeding programs. It is based upon this steady decrease in required dietary protein that feeding programs have increased the number of steps.

The 1967 and 1974 example turkey ration publications of the University of Minnesota (2,3) both employed a total of 6 rations, which for males were changed at 4 week intervals and for females after 8 weeks of age at 3 week intervals. In both the 1971 and 1977 NRC Reports (4) protein and amino acid requirements were presented for the same diet periods. Our market turkey experiments during the 1970s always employed the same intervals.

In the upper midwestern part of the U.S., corn (grain) usually costs much less than soybean meal (protein supplements), so it is desired economically to reduce the protein level in turkey rations as fast as possible. This desire was reflected in Crookston studies reported in 1964 (5) wherein protein levels were reduced every two weeks during 6-24 weeks of age. Many turkey growers who mix feed specifically for individual flocks and supply it to farms every few days have been reducing protein (and other nutrients) as fast as possible in relation to energy in order to be more competitive.

So far only age and sex have been mentioned here as modifiers of protein intake level. A relationship was observed in Minnesota studies (6) which allowed modification of protein levels based upon ambient temperature and relative costs of soybean meal and market turkey. Using the temperature portion of this research, a sample feeding program for males and females which included 5 temperature zones was presented in January, 1978 (7).

As should be expected, alternative ways of administering feeding programs have been proposed and are used widely. One of these involves the number of diet steps and questions whether there is any value in having more than 4 steps. This is illustrated by Nixey's (8) comment: "We have extremes of the Germans using 4 rations over a period when the Americans would use 9 rations. Undoubtedly, in theory the American programme should give the best fit to the bird's needs. I wonder if the bird has been informed of this fact."

Another alternative method of feeding is to feed on a pounds (set quantity) schedule (9) where so much of each feed is fed. In its simplest and earlier form the same schedule would be fed to all flocks with an expectation that the amount of consumption would serve as correction for temperature influence on feed intake. In a more modern form, there would be different schedules for birds according to season.

The research to be presented in this paper attempts to compare the dynamics involved with three feeding programs in terms of their influence on growth rate using diets where protein concentration is adequate or suboptimum. The feeding systems were (a) ration changes by age, (b) ration changes by age with adjustment for environmental temperature, and (c) ration change after consumption of a set amount of each feed. Each feeding system was used in three ambient temperature environments. As this is the first study comparing such complicated relationships, some of the conditions may have missed their target. However, the results shed interesting insights on the effect of protein supply as administered by the differing systems. Also included in this report is consideration of the influence of temperature adjusted programs for turkeys housed in alternating warm and cool environments and of a varying growth pattern which may be related to unknown environmental or infectious origins.

METHODS

The feeding systems were examined under a variety of environmental temperature conditions. The experiment (TG-803) was conducted in the Environment Building at the Agricultural Experiment Station, Rosemount. Each of four rooms (32' x 42') contains 32 pens, 4' x 8' in size. The experiment started when the Nicholas male turkeys were four weeks of age. Each pen consisted of 7 pre-weighed and pre-selected turkeys. Rooms D, C, and B were kept at constant temperatures of 45, 68, and 80°F respectively. Room A was begun at the low range of temperature at the start of the experiment and increased 5° per week until the highest temperatures were achieved during the last period (18-20 weeks of age). The 45-80 °F temperature scheme was followed excepting at 4-6 weeks of age the 45° room was held at 50-55° (due to the young age of the turkeys) and at 18-20 weeks the 80° rooms were reduced to 75-77° (due to the apparent stress of the high temperature). The actual average room temperatures are given in Table 5. Turkey body weights and pen feed consumptions were measured biweekly.

The temperature of 68° was used as the reference point around which the central feeding program was formulated. The diets were formulated for two-week ration changes. Two nutritional planes were fed in each room, designated as B and D. The B plane was predicted to be close to optimum in protein in terms of growth promoting characteristics and the D plane was suboptimum in protein. The new B diet series is shown in Table 1, which also includes for reference the lysine levels of the 1974 'B' diet series used in earlier experiments (10,11). The D diet series contained 80% of the methionine plus cystine and lysine (minimum constraints) of the B diets. Also included in two rooms were diets AA and F, which were formulated to contain 120% and 60% of the B level of these amino acids. In the AA to F series, all other critical nutrients (calcium, inorganic phosphorus, sodium, trace mineral and vitamin supplements) were at the same levels (diet compositions were identical excepting for levels of corn, soybean meal, and methionine).

When the B or D diets were temperature adjusted, all critical nutrients (methionine + cystine, lysine, calcium, inorganic phosphorus, sodium, and trace mineral and vitamin supplements) were adjusted on a therm of metabolizable energy basis; this linear adjustment corresponded to 0.5% change in nutrient concentration per 1 °F. Thus 80° diets were 6% higher and

45° diets were 11.5% lower in nutrient density than the 68° control diet. By opposite (or negative) temperature adjustment is meant that birds in the 45° environment were fed a diet adjusted for 80° and birds in the 80° environment were fed a diet adjusted for 45°.

Turkeys given the set quantity feeding system in the 45°, 80° and increasing temperature conditions all received the same amount of each ration. This amount was determined by monitoring the feed consumption of birds in the 68° room. By successive daily weighings and calculation of feed consumption, it was possible to extrapolate to the time the rations in the colder and warmer rooms should be changed.

The diets in this experiment were kept simple. They were composed of corn, soybean meal (dehulled), supplemental methionine, minerals and vitamins. No added fat, sources of unidentified factors, or antibiotics were included. As the corn and soybean meal were from blended batches, nutrient intake (total and available amino acids, true metabolizable energy) could be measured following determination.

GROWTH AT VARYING TEMPERATURES

The growth data for turkeys given different feeding systems in varying temperature environments are shown in Table 2. Considering the response of AA, B, D, and F diets (averaging the 68° and 45 to 80° environments), there was a curvilinear response in body weight to increasing protein, i.e. 2.21 kg between birds fed diets F and D, 0.80 kg between D and B, and 0.34 kg between B and AA. Statistically, the weight intervals of F to D and D to B were different at all ages; those between B and AA were significant ($P < .05$) through 12 weeks but not thereafter.

Growth was poorer at 80°, particularly after 12 weeks of age, as compared to that at 68°. Birds on the temperature adjusted system (both B and D diets) were ahead of the age and quantity system by 8 and 12 weeks of age. The effect on growth of the 6% increase in critical nutrients in the temperature adjusted diets was evident quickly, whereas with the set quantity system the increase in effective nutrient intake was cumulative in relation to decreased consumption of each feed. The days when feeds were changed with the quantity system are given in Table 3. For B and D diets at 16 and 20 weeks of age, both the adjusted and quantity systems were ahead of the age system. At the 20 weeks weighing (B series), the adjusted birds were much ahead in body weight; due to abnormally high mortality between 16 and 20 weeks of age in that treatment (the surviving birds were heavier in weight).

Birds housed at 45° were much superior in body weight to birds in the other environments. Temperature-adjusting the B and D diets (reduction of 11.5% in critical nutrients) resulted in decreased performance. The level of nutrients in the B diet were not adequate and D was deficient. It is apparent that for cool temperature adjustment to be successful, the reference diet must be optimum. That the AA diet outperformed the B diet confirms the deficient nature of the B diet. Turkeys on the quantity system performed similarly to those on the age system until 12-16 weeks of age, but by 20 weeks in both the B and the D series they were falling behind; doubtless this was due to the greater food consumption in the cool environment and consequent changing to

the lower protein diets at an earlier age (Table 3).

Turkeys in the rising temperature situation did not show as large effects to diet adjustment or quantity administration as did those in the constant warm or cold environments. At eight weeks of age, the effects were similar to those of the constant cool environment, but by the end of the study all differences were small (B and D series). Under the conditions of rising temperatures, there was a reversal of adjustment and quantity effects which resulted in a negligible effect at 20 weeks of age.

The results described in this section seem to agree with theoretical arguments put forth for both the temperature adjustment and the set quantity feeding systems. They provide support to the general objective of learning to quantitate nutrient requirements during the long growing period of the turkey. This is believed so as evidenced by the extremely sensitive responses shown to dietary manipulations in this study. No doubt one can obtain impressive performance with a few high and large nutrient intake steps, but if the turkey does have sensitive and changing needs with time and if the nutrients related to protein are more costly surely the efforts to learn the requriements over short intervals are worthwhile. This is particularly true when one formulates diets according to environmental and economic conditions, e.g. considering ambient temperature and the relative costs of energy and protein.

ALTERNATING HOT AND COLD ENVIRONMENTS

A unique characteristic of a temperature adjusting feeding system is the possible capacity to respond to short term weather expectations with least cost diets. To aid in assessing this possibility birds were alternated between 45 and 80° rooms every two weeks and fed B or D diets which were adjusted positively or negatively for temperature. The data are given in Table 4.

In the initial 2 week period at 50°F, the positively adjusted group (B diet for 50°, lysine/T = .473) gained 52.6 grams per bird per day while the negatively adjusted group (B diet for 80°, lysine/T = .551) gained 55.7 grams or 3.1 grams more. This could be expected as the B diet was not capable of supporting maximum growth at this time and the B50 diet was 9% under the B68 diet in lysine (B68 lysine was .520 lysine/T). In the next period at 80° the birds fed the adjusted B80 diet gained 10.1 grams more per bird per day than those fed the diet with opposite adjustment (B45). In the cool environment during 8-10 weeks, the oppositely adjusted B diet birds again gained more than the adjusted birds, but again the B diet was suboptimum. Thereafter the turkeys fed positively adjusted diets always gained more than those fed negatively adjusted diets. Overall, the positively adjusted birds in the B series weighed 0.57 kg more at 20 weeks of age than the negatively adjusted birds.

In the alternating temperature study conducted with the D diets, the turkeys were continually in the deficient mode regardless of the adjustment. In the hot environment the positively adjusted diet always produced better daily gains than the negatively adjusted diet. In the cool environment, where negative adjustment represented a correction of low dietary protein, greater gains were always realized from the negatively adjusted treatment excepting

in the final 18-20 week period. At 20 weeks of age, turkeys of the positively adjusted treatment were ahead by 0.12 kg: this relatively small difference resulted from opposing effects of the treatments on growth in the alternating periods.

The results of the short term temperature adjustments can be related to protein level. In the hot environment (both B and D diets), the additional protein in the temperature adjusted groups was always helpful. With the B diet, the adjustment wasn't helpful until after 12 weeks of age; because, until that time the deficiency of the B diet in the cool environment resulted in poorer performance which was correctible by the higher protein negatively adjusted diet. After 12 weeks of age, when the B diet was more sufficient, the positively adjusted turkeys forged ahead; this tends to confirm the capacity of the positive temperature adjustment to be beneficial when protein is not limiting.

EXCEPTIONAL GROWTH PATTERN

The average daily gains for two weeks periods in this study fluctuated more than would be expected with steadily gaining turkeys. Table 5 summarizes the gains of turkeys consuming the B diet (age system) in each of the rooms. Gains increased steadily until 10 weeks of age, then decreased and increased alternately. The gains during 12-14 weeks of age were particularly large. The fluctuating gains were unexpected. It was at first thought that weighing or scale errors might be responsible. It is now believed, however, that the fluctuating gains did occur.

An attempt was made to relate the gains to uncontrolled environmental influences such as relative humidity, outside air temperature, and dewpoint. Room temperatures were close to those intended and minor variations could not explain the consistent gain pattern seen in all rooms. Outside air temperature and dewpoint data are presented in Table 5 along with recorded room relative humidities. During the experimental periods there were great changes in outside temperatures and dewpoints which seem to correspond to the fluctuating gains. There was a postiive relationship between the periods of better growth and lower outside temperatures and dewpoints.

It is of interest to note that the room relative humidities increased from about 45% during 4-6 weeks to 66% during 12-14 weeks, and then decreased. The rooms were not greatly different in relative humidity during a given period although their temperatures were considerably different. That room relative humidity did not appear to relate to incoming air but did relate to the age and growth rate suggest that the ventilation system was not capable of moving enough moisture.

Relative humidity was measured daily using a sling psychrometer. It was surprising to see how high the relative humidities were, especially in the warm room. In other years it had been assumed that the humidity was too low in the warm room in view of the presence of dust, aspergillis infection, and poor growth.

Litter moisture was determined at 10 and 19 weeks of age. It was approximately 39% in the cool room and 21% in the warm room. This difference

existed although the relative humidities were similar The dewpoints in the rooms related much more to the temperatures than did the relative himidities. At the same 66% relative humidities, the 80, 68, and 45° rooms would have dewpoints of 67, 55, and 34°, respectively. It seems paradoxical that the litter moisture should be lower where the dewpoint is higher, but the amount of litter moisture is also affected by the moisture evaporating qualities (e.g. temperature) of the air.

The study of these various environmental factors has not indicated any clear cause for the exceptional gain pattern. It is assumed that the heated incoming air was of a lower humidity when the dewpoint of the outside air was low. Probably the increased metabolic activity as the birds became larger and gained more weight accounted for the increased relative humidity. Perhaps the finding of turkeys gaining very much more during 12-14 weeks of age at a 66% relative humidity resulted from an improved overall capacity to remove moisture and body heat due to the drier incoming air.

Another explanation for the growth variations may relate to the presence of subclinical infection(s) in the flock from time to time.

SUMMARY

In a warm 80° environment, temperature adjustment of a ration for male large white turkeys during 4-20 weeks of age enabled a more rapid correction of protein deficiency than a set quantity of each ration feeding system, which required a time delay prior to correction. In a cool 45° environment, temperature adjustment resulted in reduced growth because the protein level was marginal; the set quantity system did not depress growth at first, but due to greater feed consumption the subsequent lower protein diets were fed at an earlier age resulting in poorer growth.

When turkeys were switched between cool and warm environments at biweekly intervals, those given positive dietary temperature adjustment gained better in the warm environment than those which were oppositely adjusted. In the cool environment, turkeys fed negatively adjusted diets gained better whenever protein was deficient, but not when protein was adequate.

While it was expected that turkeys would gain at a fairly constant rate during two week intervals from 8 to 20 weeks of age, there were alternating periods of greater or lesser gain. While a hypothesis that variable gains were caused by changing outside air conditions is attractive, there might have been other causes such as the presence of subclinical infection in the flock.

ACKNOWLEDGEMENT

The early discussions of the concepts with Dr. M. E. El Halawani, the careful daily work of Jennifer Grabau, and the control of the environments by Manley Tollerud are recognized and gratefully acknowledged.

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Table 1. Ingredient and nutrient composition of B series diets in Experiment TG-803, with comparison to 1974 B series

	Age in weeks							
	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20
Ingredient, %								
Ground yellow corn	53.20	56.68	60.25	63.91	67.67	71.53	75.50	79.60
Soybean meal, dehulled	42.97	39.68	36.30	32.82	29.25	25.57	21.79	17.89
DL-methionine (99%)	0.053	0.027						
Defluorinated phosphate	2.50	2.36	2.21	2.06	1.91	1.75	1.59	1.42
Calcium carbonate	0.59	0.58	0.57	0.56	0.54	0.53	0.52	0.51
Salt	0.37	0.37	0.37	0.37	0.37	0.36	0.36	0.36
Trace mineral mixture (MN-74)*	0.088	0.085	0.081	0.077	0.073	0.069	0.065	0.060
Vitamin mixture (MTS-74)*	0.235	0.225	0.214	0.204	0.193	0.181	0.170	0.158
Nutrient composition								
Crude protein, %	25.57	24.26	22.91	21.54	20.14	18.60	17.21	15.68
Metabolizable energy, kcal/kg	2873	2912	2952	2993	3035	3077	3121	3167
Lysine, % per 1000 kcal ME	0.52	0.48	0.44	0.40	0.36	0.32	0.28	0.24
Met. + cys**, "	0.30	0.275	0.250	0.234	0.218	0.202	0.186	0.170
B levels in 1974***								
Lysine, % per 1000 kcal ME	- - 0.490 - -		- - 0.412 - -		- - 0.324 - -		- - 0.251 - -	
Met. + cys., "	- - 0.304 - -		- - 0.250 - -		- - 0.204 - -		- - 0.167 - -	

* Vitamin and trace mineral mixtures are given in reference 6.

** Minimum constraints for methionine and cystine in successive periods starting at 10 weeks of age were 0.225, 0.200, 0.175, 0.150, and 0.125.

*** Nutrient levels in suggested rations in references 3 and 6, and of B diets in references 10 and 11.

Table 2. Growth of turkeys fed varying nutritional levels according to age, age and temperature, and set quantity*

Temperature °F	Diet	System	Body weight in kg at week 8	12	16	20
68	AA	Age	2.72	5.33	8.57	11.75
	B	"	2.55	5.11	8.45	11.52
	D	"	2.28	4.66	7.58	10.50
	F	"	1.99	3.92	5.96	8.10
80	B	Age	2.44 a	4.74 a	7.26 a	9.11 a
		Quantity	2.48 ab	4.93 ab	7.52 a	9.22 a
		Temp.adj.	2.56 b	5.14 b	7.57 a	9.96 b
	D	Age	2.14 a	4.19 a	6.38 a	8.03 a
		Quantity	2.14 a	4.24 a	6.70 a	8.59 a
		Temp.adj.	2.19 a	4.39 a	6.60 a	8.41 a
45	B	Age	2.66 a	5.38 ab	8.85 a	12.62 a
		Quantity	2.60 ab	5.55 a	8.80 a	12.28 a
		Temp.adj.	2.53 b	5.21 b	8.54 a	12.06 a
	D	Age	2.42 a	5.12 a	8.52 a	12.11 a
		Quantity	2.39 a	5.15 a	8.34 a	11.85 a
		Temp.adj.	2.35 a	4.97 a	8.31 a	11.64 a
45 to 80	AA	Age	2.66	5.59	8.89	11.53
	B	Age	2.58 a	5.33 a	8.61 a	11.17 a
		Quantity	2.59 a	5.43 a	8.80 a	11.23 a
		Temp.adj.	2.53 a	5.30 a	8.64 a	11.32 a
	D	Age	2.38 a	5.06 a	8.04 a	10.60 a
		Quantity	2.40 a	5.04 a	8.08 a	10.57 a
		Temp.adj.	2.32 a	4.93 a	7.94 a	10.49 a
	F	Age	2.16	4.32	6.78	8.58

* Statistical differences are shown only among values in groups of 3 where letters are shown. A common letter within such a group of 3 indicates that values sharing that letter are not different at $P < .05$.

Table 3. Age (days) when ration changes were made for the set quantity system

Room temperature (°F)	Diet intervals, weeks							
	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20
	- - - - - - - - day when ration commenced - - - - - - - -							
Age reference	28	42	56	70	84	98	112	126
B diet:								
45	28	41	54	66	80	92	106	118
80	28	42	58	73	87	103	120	- -
45-80	28	41	54	66	80	93	107	120
D diet:								
45	28	41	54	66	80	92	104	116
80	28	42	59	74	90	107	127	- -
45-80	28	41	54	66	79	92	106	119

Table 4. Growth of turkeys fed positive temperature adjusted or negative (opposite) temperature adjusted diets in alternating hot and cold environments

Dietary temperature adjustment	Body weight (kg) at week							
	6	8	10	12	14	16	18	20
B diets:								
Positive	1.44	2.47	3.73	4.97	6.87	7.88	9.76	10.21
Negative	1.49	2.38	3.75	4.83	6.72	7.55	9.36	9.64
	Av. daily gain (gm/bird), + weeks of age and temperature (^{o}F) for period							
	4-6 52	6-8 80	8-10 45	10-12 80	12-14 45	14-16 80	16-18 45	18-20 75
Positive	52.6	73.9	89.8	89.1	135.8	72.6	134.6	44.5
Negative	55.7	63.8	97.9	77.3	135.1	59.1	129.2	42.8
Positive over negative	-3.2	+10.1	-8.1	+11.8	+0.7	+13.5	+5.4	+1.7
	Body weight (kg) at week							
	6	8	10	12	14	16	18	20
D diets:								
Positive	1.33	2.28	3.23	4.54	5.70	7.09	8.11	9.38
Negative	1.26	2.26	3.17	4.61	5.72	7.14	8.08	9.26
	Av. daily gain (gm/bird), + weeks of age and temperature (^{o}F) for period							
	4-6 80	6-8 45	8-10 80	10-12 45	12-14 80	14-16 45	16-18 80	18-20 45
Positive	44.3	67.8	67.6	93.6	83.0	99.2	73.6	91.1
Negative	39.6	71.0	65.5	102.8	79.8	101.2	71.8	85.9
Positive over negative	+4.7	-3.2	+2.1	-9.2	+3.2	-2.0	+1.8	+5.2

Table 5. Biweekly gains of large white male turkeys (B diets) under varying environmental conditions

Measurement and room	Age in weeks with experimental dates							
	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20
	12/3	12/17	12/31	1/14	1/28	2/11	2/25	3/11	3/25/81
Average daily gain, gm/bird/day								
68 °F	53.9	78.6	93.5	89.8	127.8	110.6	126.9	92.7
80	52.2	72.4	80.7	83.7	100.0	80.0	79.9	56.8
45	55.0	84.0	102.1	92.4	144.5	103.7	130.3	139.0
45 to 80	53.0	82.6	98.4	98.0	128.2	106.2	114.0	70.4
Average room temperature, °F								
68 °F	67.2	67.0	68.3	67.3	67.4	67.8	69.7	69.1
80	78.2	79.4	80.6	79.7	80.1	79.8	80.2	77.0
45	52.8	45.5	45.8	46.9	46.7	48.8	46.3	50.8
45 to 80	52.2	50.2	54.1	60.4	63.8	69.2	76.7	76.3
Average room relative humidity, %								
68 °F	44	56	57	61	64	64	67	50
80	38	49	58	66	66	67	61	50
45	46	50	54	56	64	65	61	46
45 to 80	52	52	62	63	68	60	55	52
Average litter moisture, %								
63 °F				22.8				32.6
80				19.3				23.4
45				38.7				38.8
45 to 80				29.8				34.1
Average outside								
Temperature, °F	23.8	16.8	10.1	26.1	8.4	36.0	30.0	39.1
Dew point, °F	13.9	6.1	-.2	14.6	-1.5	25.9	18.4	19.6

REGULATION OF AVIAN DIGESTION

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We have studied digestive function of turkeys in our laboratory for nearly 15 years and we've described many aspects of gastrointestinal (GI) contractile activity (e.g. Duke et al., 1969b; 1972; 1975a and 1975b; Lai and Duke, 1978), intestinal absorption (Duke et al., 1969a; Sudo and Duke, 1980) and nutrient digestibility (Duke et al., 1970). We have recently been most interested in how digestive processes are regulated and have looked at neural mechanisms (Duke et al., 1977), diurnal cycles (Duke and Evanson, 1976) and hormonal regulation (Duke et al., 1979; 1981; Savory et al., 1981). We're all familar with reproductive hormones, but hormones are involved in regulating many bodily functions. The largest group of hormones is those involved in regulation of the digestive tract. GI hormones include gastrin, secretin, motilin, gastric inhibitory peptide (GIP), cholecystokinin (CCK), and pancreatic polypeptide (PP). We've studied only the latter two and mostly we've concentrated on PP.

Pancreatic polypeptide was discovered in the chicken pancreas by Dr. Joe Kimmel of the University of Kansas Medical Center in 1971 (Kimmel et al., 1971; 1975). PP is believed to be a hormone, like insulin and glucagon which also originate in the pancreas, and to be involved in regulation of GI activities. Both the discovery and research on hormones has become a matter of intense interest in many labs because of the development of very sensitive radioimmuno assays (RIA) for hormones. Because Dr. Kimmel is a Biochemist and because he had little experience in working with poultry, he contacted me in 1974 to work on research aspects requiring either an avian or a GI physiological background. Studies in our labs have provided significant insight into the role of APP in chickens and turkeys. Dr. Kimmel and his collegues initially found that APP was released into the bloodstream after eating indicating that it must be involved in the digestive processes somehow (Kimmel et al., 1971). Next they found that injections of APP caused an increased flow of gastric secretions and an increase in the concentration of acid and pepsin in the secretions (Hazelwood et al., 1973). They also found that the hormone had no effect on pancreatic or biliary secretion and no effects on the cardiovascular system.

In my first collaborative study with Dr. Kimmel I surgically implanted strain gauge transducers on the proventriculus, gizzard, duodenum, ileum, one cecum, and the colon to permit detection of contractions in these organs (Duke et al., 1979). After recovery from surgery, turkeys were connected to an electronic recorder, to record the contractile activity, and APP was intravenously injected. The motility of the proventriculus, gizzard and duodenum were significantly depressed by the APP injections while the motility of the ileum, cecum and colon were much less depressed although still depressed.

Thus, at this point we knew that eating causes a release of APP into the blood, APP stimulates secretion of digestive juices and inhibits motility. The former promotes increased digestion while the latter would slow passage of food through the intestinal tract which also allows digestion to be more thorough and promotes greater utilization of the diet.

At this time, during a visit by John Savory of the Poultry Research Centre in Edinburgh, Scotland, we found that injections of another hormone cholecystokinin (CCK), which is found in intestinal tissues, also depresses GI contractility. CCK is also released after eating and it is believed to be involved in satiety. Perhaps by slowing gastric emptying a renewal of desire to eat is delayed.

In our most recent work we were interested in determining what nutrients, or combination of nutrients in a meal, are responsible for the release of APP and in what portion of the gut is the presence of these nutrients "sensed" so that a reflex release of APP might occur.

Turkeys were surgically fitted with devices which would allow us to isolate the stomach, duodenum or upper ileum from the rest of the tract and then to inject solutions into the isolated gut segment (Duke et al., 1981). The following solutions were injected: HCl, NaCl, amino acids, glucose and corn oil and a small balloon was inflated to simulate mechanical stimulation of the gut -- the solutions provided chemical stimulii. Blood samples taken before introducing one of the solutions or the balloon and at 3, 10, 20, 30 and 50 minutes after the introduction, were analyzed for APP content. Amino acids appeared to be the best substance at stimulating APP release, HCl was the next best (Table 1). The stomach was the site in which stimulii were most likely to stimulate APP release (Table 2). The ileum was next most responsible and the duodenum was least responsive.

Thus diets with higher protein content should cause greater APP release and APP should cause slower gastric emptying. This is generally what has been observed in determinations of total transit time of diets.

These results may be of practical significance in treatment of GI diseases or in improving utilization of diets. Injections of APP could be used to depress motility during diarrhea; better yet, do this by feeding of appropriate materials to stimulate APP release. Other hormones, yet to be tested, might be used to stimulate appetite during anorexia. Use of hormones to slightly depress appetite (CCK) or to increase digestibility of the diet (e.g., slow gut contractile activity or increase secretion of gut enzymes such as occurs with APP) may improve efficiency of utilization of diets. Hormones might be found which have very specific aspects that could be of practical value, e.g., slowing of cecal emptying to promote greater bacterial digestion of fiber, or producing cecal emptying just prior to processing to help prevent contamination of carcasses with cecal contents.

As indicated at the onset, research on GI hormones is expanding very rapidly. Functions of known hormones are being elucidated and new hormones are being discovered. Finding practical applications for such a rapidly expanding knowledge base is an exciting challenge. Unfortunately only three laboratories in the world have ongoing research on avian GI hormones; only our lab is studying the relationships of hormones to GI motility.

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Table 1: Mean (± Standard Deviation) integrated plasma responses [(ng/ml)min] (total amount of APP released following treatment) of (APP) following five treatments. Averages were calculated over all gut sites. Means over the same line are not significantly different (P=0.05).

Treatments				
Glucose	NaCl	Corn Oil	HCl	Amino Acid
20.90	42.06	68.50	159.60	201.60
(±39.54)	(±38.54)	(±79.58)	(±130.86)	(±168.08)

Table 2: Mean (± Standard Deviation) integrated plasma responses [(ng/ml)min] of [APP] in three gut sites for all treatments at that site. Means above the same line are not significantly different (P=0.05).

Gut Sites		
Duodenum	Ileum	Stomach
46.24	79.76	169.56
(±43.50)	(±92.06)	(±165.40)

APPLICATION OF RECENT RESEARCH IN NUTRITION TO FEED FORMULATION IN TURKEYS

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In this presentation, the results from recent research in nutrition of turkeys conducted at our university will be presented. These experiments include investigations involving protein, methionine and lysine requirements, deficient amino acids, dehulled soybean meal digestibility, feed efficiency improvement from fat, energy evaluations, inorganic sulfate, trace minerals, and antibiotics. These experiments were conducted by a team of researchers including at least six graduate students who are cited in the references. Brief description of the experiments, results and conclusions are presented. An attempt to provide an interpretation and application of the results to commercial production of turkeys has been made.

PROTEIN, METHIONINE AND LYSINE REQUIREMENTS

A series of experiments (1,2) have been conducted to determine the methionine and protein requirements of growing turkeys. In these experiments, it was reported that the Medium White turkey requires about 30.3% protein at one day of age and its protein needs decrease at a rate of .61 and .78 percentage units per week for males and females, respectively.

In an experiment with Large White turkeys (3), the protein requirements at 10, 14 and 18 weeks of age were estimated to be 21.3, 19.5 and 17.6% for males and 21.7, 18.4 and 15% for females, respectively. In a more recent experiment (4), protein requirements of Large White turkeys was found to be about 23.1% at 8 weeks of age and decreases to 17.2% at 20 weeks for males. In females, the protein requirement was 22.3% at 8 weeks of age and decreases to 15.8% at 20 weeks of age. In each case, the protein requirement is believed to decrease in a linear manner with age.

In the first series of experiments (1,2), the requirements for total sulfur amino acids were reported to be approximately 1.10% from 0 to 4 weeks of age, 1.00% from 4 to 8 weeks of age, .93% from 8 to 12 weeks of age, and .75% from 12 to 16 weeks of age by Medium White turkeys. Results from experiments (5) with Large White turkeys from 0 to 7 weeks of age indicate that the requirement for total sulfur acids is in excess of 1.1% to obtain optimum body weight gains and approaching 1.2% to obtain optimum feed efficiency.

In a study (3) conducted to determine the need for added lysine in a corn-soybean meal diet supplemented with large amounts of methionine, the minimum lysine requirements of Large White turkeys were about 1.4, 1.2 and 0.9%, respectively, or 4.5, 3.7 and 3.0 g/kcal metabolizable energy. It was concluded that another amino acid appeared to be equally or more deficient than lysine in diets composed primarily of corn and soybean meal with 0.4% added methionine.

DEFICIENT AMINO ACIDS

Three experiments were conducted (6) with young turkeys between 1 and 3 weeks of age to determine which amino acids are deficient in a 22% protein corn-soybean meal diet supplemented with .3% DL-methionine. The body weight gains of turkeys fed a 30% protein corn-soybean meal diet and a 22% protein diet supplement with 14 individual essential amino acids were equivalent but much greater than that from the unsupplemented 22% protein diet. By removing valine, lysine, threonine, or isoleucine from the fully supplemented diet (22% protein plus 14 added amino acids), body weight gains were significantly reduced indicating that these amino acids are deficient in a 22% protein corn-soybean meal diet for young turkeys.

In another three experiments (7), a 21% protein diet was supplemented with .30 or .60% lysine, .64% arginine, .44% valine or .31% threonine in all possible combinations to form a 3 x 2 x 2 x 2 factorial design. Each of the 24 diets plus a 29% protein diet was fed from 4 to 6 weeks of age. From feeding the unsupplemented 21% protein diet, the fully supplemented diet and the 29% protein diet, average body weight gains were 772, 848 and 859 g, respectively. Body weight gains were significantly increased from 800 to 815 or 825 g by adding .30 or .60% lysine ($P<.01$), from 803 to 824 g by adding arginine ($P<.01$) and from 804 to 823 g by adding valine ($P<.05$). Results indicate that lysine, arginine and valine are deficiency amino acids in a 21% corn-soybean meal diet supplemented with methionine for young turkeys between 4 and 6 weeks of age.

From these studies with variable protein and amino acids in diets containing primarily ground yellow corn and dehulled soybean meal, diets for turkeys should first be formulated to meet the total sulfur amino acid requirement and then the protein requirement. Because other amino acids are equally or more deficient than lysine in such diets, the diets should be formulated for protein needs and then possibly lysine needs. The protein content of an ingredient can be determined easily at less cost and with greater accuracy than its lysine content. Because of this advantage, the protein of the commercial turkey diet should be determined on the basis of protein content rather than lysine content. If large amounts of corn gluten meal and other low-lysine containing ingredients are used in commercial diets, then the formulation of the diets on lysine needs before considering the protein needs may be justified.

DEHULLED SOYBEAN MEAL DIGESTIBILITY

An experiment was conducted (8) to determine the apparent digestibilities of dry matter, protein, amino acids, ether extract, fiber and nitrogen-free extract of dehulled soybean meal in adult male turkeys. Average apparent dry matter digestibility of dehulled soybean meal was only 53.5%. The protein was 87% digested, its amino acids 80 to 93% digested and the ether extract 89% digested. However, its crude fiber and nitrogen-free extract were only 14 and 4% digested, respectively. The poor digestibility of its nitrogen-free extract, which makes up about 30% of dehulled soybean meal, accounts for its comparative low metabolizable energy in contrast to ground yellow corn.

METABOLIZABLE ENERGY EVALUATIONS

A study (9) has been conducted to determine the effects of the correction of excreta energy to nitrogen balance in the determination of true metabolizable

energy (TME) by the Sibbald procedure. Data have been obtained which support the hypothesis that the metabolic fecal and endogenous urinary energy as measured from the fasted birds provide a greater correction than justified for an accurate TME determination. Fasted birds were found to have greater nitrogen losses from non-dietary sources than fed birds have. Thus, fasted birds have greater nitrogen losses from non-dietary sources than do fed birds. The results of this study provide evidence to question the reliability of the TME determinations. The difference between the values for TME and TME_n in this study explains in part the previously reported abnormally high values (in my opinion) for TME over values for the metabolizable energy corrected for nitrogen retention obtained in comparable ingredients.

STABILIZED FAT

Two experiments have been conducted during the past two years with stabilized fat as a variable in diets of Large White turkeys between 8 and 20 weeks of age. Each of two fats varying in linoleic acid was added at the 0, 5 and 10% levels to diets containing 18, 20, 22 and 24% protein. On the average, body weights were increased .77%, feed consumption decreased 1.04% and feed efficiency increased 1.93% for each 1% added fat. Feed efficiency increased 1.8, 2.0 and 2.5% for each 1% added fat during the 8 to 12, 12 to 16 and 16 to 20-week period, respectively. Fats containing 15 and 32% linoleic acid failed to produce differences in feed efficiencies at any age period (4).

These results confirm those of our previous studies (Poultry Science 53: 2072-2082, 1974). In this earlier study, feed efficiencies increased about 1.25 to 1.64% for each 1% added fat at 10 weeks of age and 2.23 to 2.52% for each 1% added fat to 20 weeks of age. Thus, between 10 and 20 weeks of age, feed efficiencies increased 2.94 to 3.05% for each 1% added fat. In all of these studies, fat was added to the diet with soybean meal in the place of ground yellow corn to hold protein constant. These results illustrate clearly that high fat diets provide very economical sources of energy even when fat is priced three times that of ground yellow corn and turkeys are between 11 and 20 weeks of age.

EXPERIMENTS WITH SULFATES

Two experiments (10) have been conducted to determine if the addition of sulfate compounds to the diet of young turkeys could reduce the need for added methionine. All diets contained 48% ground yellow corn, 36% dehulled soybean meal and 12% meat and bone meal. Methionine was added at the 0, .05, .10 and .15% levels, and sulfate was added at the 0, .095 and .190% levels from sodium sulfate or at the 0, .118 and .235% levels from potassium sulfate to form a 4 x 6 factorial design of 24 dietary mixtures. From feeding diets containing 0, .05, .10 and .15% added methionine, 7-week body weights were 2020, 2162, 2234 and 2243 g, respectively. Body weight were increased 7.0, 10.7 and 11.1% from the addition of .05, .10 and .15% added methionine. From feeding diets containing 0, .0215 and .0430 sulfur from the sulfate compounds, body weights were 2163, 2166 and 2167 g, respectively (2162 and 2172 from adding sodium sulfate or potassium sulfate). No significant interactions among the factors under study were observed in this study indicating that the corn-soybean meal diet with 12% meat and bone meal had sufficient sulfur to meet the sulfur needed other than for methionine and cystine.

EXPERIMENTS WITH MENHADEN FISH MEAL, SELENIUM AND MOLYBDENUM

A large part of the increase in growth from adding menhaden fish meal to diets of young turkeys has been shown to be due to the selenium content of the fish meal (11). In further experiments (12), the addition of 5% menhaden fish meal to diets of young turkeys containing .2 ppm added selenium also increased body weights 5.0% ($P<.001$) indicating the presence of another growth factor other than selenium in menhaden fish meal. In a recent unpublished study (13), additional data were obtained to support these findings, and the addition of 5 ppm molybdenum to the diets failed to increase growth indicating that molybdenum is not the unidentified growth factor in menhaden fish meal.

ANTIBIOTICS

From a large number of experiments conducted with added antibiotics to diets of turkeys to 7 or 8 weeks of age at our university, an increase in body weights was observed in 52 of the 58 experiments. The average increase in body weights was 2.7%. In over one-half of the experiments, the increase in growth was in excess of 4%. During the past two years, four additional experiments (14) involving 192 pens of turkeys have been conducted with bacitracin added at 50 grams per ton as a variable. Similar increases in body weight were observed as in the previous experiments.

The growth response from antibiotics is greatest at two or four weeks of age when expressed on a percentage basis. The majority of the absolute increase is usually obtained when turkeys are 10 to 12 weeks of age and is maintained until market age or until maximum weekly gain is reached. Thus, under comparable conditions, antibiotics are as effective in increasing growth rate of turkeys today as ten or twenty years ago.

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MINNESOTA RESEARCH ON BONE DEVELOPMENT

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This report summarizes a number of investigations which have been conducted by the authors.

I. "FIELD RICKETS"

"Field rickets" is a condition of very young poults (5-30 days), and is recognized by reduced activity and leg weakness. The incidence of "field rickets" in affected flocks is usually around 20%. In severely affected poults, the bones are soft and fragile. The finding of soft bones, coupled with radiographic, microscopic and biochemical evidence, points to defective mineralization of the rapidly growing bones. However, analyses of feed samples from affected flocks indicate that normal levels of calcium and phosphate are present in the ration.

Other possible factors in the development of "field rickets" include deficiency of vitamin D, and malabsorption. There is evidence that vitamin D metabolism in affected poults is abnormal, and this line of investigation is being pursued. We have also made an association between the occurrance of "field rickets" and reovirus enteritis. Enteritis may impair absorption of the nutrients which are required for formation of bone.

II. BONE DEVELOPMENT AND DEFORMITIES IN TURKEYS

Bones from turkeys from 8 to 39 weeks of age were supplied to us by Nicholas Turkey Breeding Farms. These bones were collected from normal turkeys from five developmental lines. Our interest was to establish patterns of normal development of bones of turkeys. Examination of these bones yielded a bank of reference data of lengths, photographs, and radiographs which we can use to evaluate cases of field problems.

This study also yielded information about tibial dyschondroplasia (TD), a common abnormality in turkeys. TD was present in 80% of the toms at 10 weeks of age. Many of these lesions were mild, and the incidence dropped steadily until the toms were about 22 weeks of age. Beyond that age, 5-15% of the toms examined had TD. More importantly, 6% of toms from 22-38 weeks had visible deformities of the tibiotarsus, and all of these deformities were associated with TD. The heaviest of the five

lines had the highest incidence of TD, and the highest incidence of deformities (25%). The peak incidence of TD in the hens was 65%, at 13 weeks of age. There was no TD found in hens beyond 21 weeks and there were no deformities.

The findings of a high incidence of TD in young toms, and an association between TD and deformities in older toms leads us to reconsider the significance of the lesion. Mild TD in toms may be of no significance to birds less than 18 weeks. However, a high incidence of TD in a flock of growing toms may be an indicator of future deformities. Severe TD is still regarded as a cause of leg problems.

III. EFFECT OF DIETARY FLUORIDE ON THE PERFORMANCE OF TURKEYS

Large white tom turkeys were fed a basal corn-soybean ration with tricalcium phosphate which contained 10 ppm of fluoride. Sodium fluoride was added to provide added fluoride levels of 12.5, 25, 50, 100, 200, 400, and 800 ppm. An additional diet incorporated defluorinated phosphate and no additional fluoride. The poults were weighed and samples were collected at 2, 4, 8, 12, and 18 weeks.

Poults on levels of fluoride of 200, 400, or 800 ppm had reduced growth rates. Treatments of 12.5 to 100 ppm added fluoride had the highest market weights, and the weight of the poults fed a control diet containing defluorinated phosphate was similar to these. The weight of the poults on the unsupplemented tricalcium phosphate ration containing no added fluoride was slightly less.

IV. EFFECT OF PHOSPHATE SOURCES ON EARLY PERFORMANCE OF TURKEYS

Large white turkey poults were started and maintained for two weeks on diets using phosphate supplements from each of 43 sources. The basal diet contained 0.44% phosphorus, and diets were formulated to contain 0.65% phosphorus and 1.2% calcium. The parameters of this trial include weight gain, feed consumption, leg score, and bone ash. Bones will also be examined microscopically, and compared with those from poults with experimental phosphate deficiency.

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LEG WEAKNESS IN TURKEYS: A REPORT ON CURRENT RESEARCH

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Leg weakness problems have afflicted the turkey industry for a number of years. A survey conducted in 1952 by Nicholas Turkey Breeding Farms reported that 10% of the heavy strains of toms showed some degree of leg abnormalities (Nicholas Turkey News, 1979). Progress has been slow in eliminating this problem from turkey flocks because most investigators agree that a decrease in the incidence of leg deformities has not occurred since this 1952 report (Blair, 1981).

Most of the leg weakness problems in turkey flocks occur between 8 and 20 weeks of age (Sanger et al., 1974). Occurrences predominate in males; hens can be afflicted with leg deformities, but the incidence remains low (Glass, 1971; Buffington et al., 1975; Laing, 1976). The economic loss due to mortality, medication cost, downgrading, and condemnations was estimated in 1971 to cost U.S. turkey producers $17 million annually (Anonymous, 1971).

The causes of leg weakness appear to be varied and complex (Glass, 1971). Research, especially in the areas of pathology and nutrition, has succeeded in identifying various nutrients and disease organisms which can lead to the production of leg weakness (for a review, see Pierson and Hester, 1981). However, even after these factors have been identified and incorporated into management programs of turkey operations, the leg weakness problem still prevails (Norris, 1971).

Limited research has been conducted on the physiological, managerial, and environmental factors which may be related to leg weakness in turkeys (Pierson and Hester, 1981). As a result of this inadequacy of information, our laboratory has emphasized these areas of research. In one of our first investigations (Pierson et al., 1981b), physiological comparisons were made between toms with and without leg weakness. No differences were found between normal and lame turkeys with respect to packed cell volume, hemoglobin concentration, and the plasma levels of uric acid, inorganic phosphate, calcium, alkaline phosphatase activity, and testosterone. However, lame birds were found to have significantly higher plasma corticosterone concentrations and total leukocyte counts than healthy controls. Two postulations were suggested to explain the correlation between lameness and elevated levels of the corticoid. First, the rise in plasma corticosterone may be considered part of an overall stress response to leg weakness as a stressor. Food and water deprivation (Buckland et al., 1974; Saleh and Jaksch, 1977; Beuving and Vonder, 1978) or the presence of pathogen (Freeman, 1971) may have elicited the observed adrenal response in these lame turkeys. The fact that lame birds generally appeared less thrifty and less active than their pen mates may provide additional indication that some agent of stress was present. A second possibility may be that lameness is actually a consequence of increased plasma corticosterone concentrations. It is well known that corticoids may directly influence the integrity of bone and cartilage. For example, Hublé (1957) showed that cortisone acetate injections inhibit chondrogenesis and bone growth in chickens. Theoretically, increased corticosterone levels could result in structural

infirmities at sites of rapid bone growth like the tibia and tarsometatarsus, predisposing birds to leg weakness. The observed increase in total leukocyte counts of lame turkeys may reflect an acute response of the immune system to elevated plasma corticosterone concentrations since similar leukocytoses have been reported in response to ACTH or corticoid administration (Newcomer, 1957; Glick, 1961). Although the presence of a pathogen was not confirmed among leg deformities observed in this study, the increased leukocyte count may have been in response to an infectious agent (Pierson et al., 1981b).

Since corticosterone concentrations were increased in the plasma of lame turkeys, the possibility that stressors may be related to the leg weakness problem was investigated. Chronic handling, in which turkey toms were weighed at 5, 10, 15, and 20 weeks of age in addition to daily direct contact with project personnel, was evaluated as a possible inducer of leg weakness. Although plasma corticosterone concentrations were higher in the handled turkeys as compared to non-handled controls, there were no significant differences between the two groups in the incidence of leg weakness (Pierson et al., 1981b). Likewise, when high and low brooding temperatures were evaluated as possible stressors, no differences were found between these two treatment groups and a moderate brooding temperature with respect to incidence of leg weakness, feed/gain ratio, mortality, and live body weight when measured over the 20 week grow-out period (Pierson et al., 1981c).

Since chronic handling and extreme brooding temperature as possible stressors could not be correlated with leg problems, our research attention was redirected to trying to understand why toms were more susceptible to leg weakness than females. The broad breast of the turkey tom has led investigators to postulate that excessive weight causes undue pressure on the leg muscles and bones making males more susceptible to leg weakness than females (Laing, 1976). Data exist to both confirm and confute this hypothesis. Supportive evidence include the following: 1) a heavier strain of male turkeys had a greater incidence of abnormal cartilage lesions in the tibia than a lighter strain; the progeny resulting from a cross of the two strains was intermediate in body weight and cartilage formation, and 2) male turkeys whose feed was restricted by 20% between 5 and 10 weeks of age showed a reduction in body weight and incidence and severity of abnormal cartilage formation of the tibia (Steinke, 1971). Evidences to refute the postulation are as follows: 1) turkey whose feed was restricted so as to significantly reduce rate of gain showed the same incidence of leg weakness as full-fed controls (Adams and Stadelman, 1978); 2) within a medium-weight strain of turkeys, the lightest birds were just as susceptible to leg weakness as the heaviest birds (Buffington et al., 1975); and 3) a lighter strain of turkeys did not differ in leg weakness incidence from a heavier strain (Adams and Stadelman, 1978).

Another factor besides body weight which may contribute to the difference in the incidence of leg weakness between toms and hens is the sex steroids. Our laboratory has shown that there is a correlation between plasma androgen concentrations and the incidence of leg weakness in toms. Specifically, caponization resulted in an increased incidence of leg abnormalities and decreased plasma androgen concentration when compared to nonsurgical controls (Pierson et al., 1981a). It is well known that androgens play a significant role in the growth and metabolism of the skeletal system. In human males, excessive secretion of testosterone prior to puberty interferes with the attainment of full stature by accelerating the fusion of the epiphyses and shafts of long bones. In contrast, a prepubertal deficiency of testosterone may facilitate excessive elongation of bones (Sawin, 1969). Reports on the effect of caponization on the lengths of

long bones in fowl are conflicting. One investigator reported an increase in the length of the humerus, tibiotarsus, and three phalanges of the third toe of caponized Leghorns (Hutt, 1929), while another investigator reported no differences due to castration in the lengths of bones of the extremities in both normal and creeper fowl (Landauer, 1937). The addition of testosterone to in vitro cultures of embryonic fowl bone enhanced calcification and the synthesis of osteoid tissue (Puche and Romano, 1968; 1969). Some types of leg weakness in turkeys cannot be induced after the closure of the epiphyseal plate which occurs at 17 and 23 weeks of age for hens and toms, respectively (Nairn, 1969; 1973). The earlier physiological aging of long bones of females (Silberberg and Silberberg, 1971) may contribute to the lower incidence of leg weakness. If androgen concentrations could be boosted at an earlier age in toms, at least during the critical period of 8 to 20 weeks of age when leg problems become prevalent, then perhaps leg weakness incidence could be reduced by promoting earlier maturation of bone. Research is currently in progress to evaluate this possibility. To promote earlier sexual maturity, a high intensity step-up lighting program was employed as outlined in Table 1. The step-up lighting regime was compared to a low-intensity step-down lighting program commonly used by the turkey industry for environmentally controlled housing units (Table 1). The performance traits of body weight, feed/gain ratio, and mortality did not differ significantly between lighting programs (Table 2). The step-up lighting program was successful in significantly reducing the incidence of leg weakness as observed by two different evaluators (Table 2). The right tarsalmetatarsal bone was significantly shorter for birds reared in the step-up lighting program, while lighting had no effect on the width and ash content of this same bone (Table 2). Total testes weights as well as plasma androgen concentrations at both 98 or 99 and 127 or 128 days of age were significantly increased by the step-up lighting program (Table 2).

The results of this last study indicate that the use of a step-up lighting program can significantly reduce leg weakness problems in turkeys. Light stimulation reduced the incidence of leg abnormalities either through increased exercise and/or by shortening the length of long bones. It is not clear at this particular stage of experimentation which mechanism is involved or whether it is a combination of both.

Toms of the high intensity step-up lighting program were visually more active and their secondary sex characteristics of strutting, coloration of the head, etc., were more pronounced. Plasma androgen concentrations and testes weight also confirmed that birds of the step-up lighting program were approaching sexual maturity at an earlier age. Toms in the low intensity step-down lighting program spent more time resting and sitting on their hocks. As a result of the increased activity displayed by the toms of the step-up lighting program, the plumage was cleaner; the birds were more thrifty in appearance which was undoubtedly the result of spending less time in contact with the litter.

The shortened metatarsals of turkeys of the step-up lighting program may be the result of increased circulating concentrations of androgens. It is well known that androgen induces the closure of the epiphyseal growth plates which results in the shortening of long bones (Sawin, 1969). Two postulations are suggested to explain why shorter bones may contribute to a lower incidence of leg weakness. First, if shorter bones are indeed a result of earlier closures of the epiphyseal plates, then these bones may be less susceptible to pathogens which can cause lameness. Supportive evidence for this hypothesis is provided by Nairn (1969) who reported that once the epiphysis combines with the diaphysis of long bones of turkeys, that leg weakness resulting from bacterial osteomyelitis

and synovitis cannot be induced. Secondly shorter bones may provide stronger skeletal support. Although bone ash values were the same between lighting programs, the possibility exists that the ratio of the minerals or collagen and ground substance of mucopolysaccharides were not the same.

Management practices currently employed by the turkey industry to delay sexual maturity and/or decrease the activity of turkey may also delay the maturation of long bones. The fact that bones from females mature faster than males (Silberberg and Silberberg, 1971) may be one reason why hens have not demonstrated a high incidence of leg problem under current management systems. A delay in the physiological aging of long bones of turkey toms may be one of the causes of leg weakness that has plagued the turkey industry for so many years.

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Table 1. Lighting programs for turkey toms.

Lighting program	Age(days)	Hours of light	Light intensity (lux)
Step-up	1 to 3	24	10
	4 to 49	9	2.5
	50	10	20
	57	10½	20
	64	11	20
	71	11½	20
	78	12	20
	85	12½	20
	92	13	20
	99	13½	20
	106	14	20
	113	14½	20
	120	15	20
	127 to 135	15½	20
Step-down	1 to 3	24	10
	4	23	2.5
	5	22	2.5
	6	21	2.5
	7	20	2.5
	8	19	2.5
	9	18	2.5
	10	17	2.5
	11	16	2.5
	12	15	2.5
	13	14	2.5
	14	13	2.5
	15 to 135	12	2.5

Table 2. The effect of lighting programs on performance traits; incidence of leg weakness; bone length, width, and ash; total testes weight; and plasma androgen concentration.

Variable ($\bar{x}$)	Lighting Program	
	Step-up	Step-down
Body weight at 95 or 96 days of age (kg)	8.28	8.14
Body weight at 130 or 131 days of age (kg)	11.79	11.85
Feed/gain ratio from 0 to 130 or 131 days of age	2.86	2.80
Mortality from 16 to 131 days of age (%)	6.2	7.8
Leg abnormalities from 16 to 130 or 134 days of age (%)		
Evaluator 1	5.4 *	10.4 *
Evaluator 2	36.6 *	43.4 *
Right tarsalmetatarsal length (cm)	15.24*	15.70*
Right tarsalmetatarsal width (cm)	1.22	1.22
Epiphyseal and diaphyseal bone ash (%)	53.8	53.5
Diaphyseal bone ash (%)	63.6	63.9
Total testes weight at 135 or 136 days of age (g)	7.68*	6.02*
Androgen concentration at 98 or 99 days of age (pg/ml)	273*	128*
Androgen concentration at 127 or 128 days of age (pg/ml)	461*	367*

*Means in a row are significantly different between lighting programs ($P<.05$).

CALCIUM, PHOSPHORUS AND VITAMIN D DEFICIENCIES IN YOUNG TURKEYS: DIAGNOSIS AND TREATMENT

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INTRODUCTION

Leg weakness in turkeys results from several anatomical defects which are, in turn caused by nutritional, genetic and pathological factors. One such defect, easily characterized in the laboratory by the reduced percentage of bone ash, is the subject of the present communication.

Calcium, phosphorus and vitamin D deficiences, and unidentified factors which interfere with some metabolic components of the system of mineral assimilation are common causes for reduced bone calcification. Since both diagnosis and treatment, are based on biochemical and physiological aspects of the disorders, these will be summarized briefly. Recent comprehensive reviews of the general field may be found in DeLuca, 1980; Frazer, 1980; Hurwitz, 1978: Lawson, 1978; Norman, 1979, 1980.

BASIC CONCEPTS

The association of calcium and phosphorus in animal nutrition and the essentiality of vitamin D to the normal transfer of the mineral from diet to bone and for calcium homeostasis, have been recognized for many years. It is now well established that vitamin D_3, (cholecalciferol) can be synthesized in the skin from 7-dehydrocholesterol, under the influence of ultraviolet irradiation. With birds kept in confinement, where the level of ultraviolet irradiation is low, this route cannot supply any significant quantities of vitamin D, and the requirements for the vitamin must be satisfied by dietary means, and reach body pools through intestine.

Vitamin D_3 is transported in circulation bound to specific binding protein. Some of the vitamin is hydroxylated in the liver to 25-hydroxycholecalciferol, $25(OH)D_3$. This hydroxylated metabolite is distributed in the entire body but some is further hydroxylated in the kidney to 1,25 dihydroxycholecalciferol, $1,25(OH)_2D_3$, or to 24,25 dihydroxycholecalciferol, $24,25(OH)_2D_3$. In addition, other dihydroxylated and trihydroxylated metabolites are formed to which no important physiological role could be assigned so far. $1,25(OH)_2D_3$ is considered to be the hormonal form of vitamin D_3: it promotes calcium and phosphorus absorption, bone calcium mobilization, and also supports bone calcification. The role of $24,25(OH)_2D_3$ is still controversial; it is believed, by some, to be important in normal bone development, and appears to be essential for embryonic development in the chicken.

Of the vitamin D hydroxylation steps, 25-hydroxylation is apparently not influenced by mineral metabolism. On the other hand, the activity of enzyme responsible for the 1 hydroxylation step, 25-hydroxycholecalciferol-1-hydroxylase (1-hydroxylase), is feedback linked to calcium homeostasis. Its

activity is stimulated by any form of calcium deficiency, whether nutritional or metabolic, including egg shell formation in the laying bird and accelerated growth in the chick. This feedback relationship is also indicative of the hormonal nature of $1,25(OH)_2D_3$.

Upon arrival at the intestinal mucosa, $1,25(OH)_2D_3$ is bound to specific receptor molecules and taken up by the nucleus. There, its presence results in synthesis of mRNA which effects synthesis of proteins such as calcium-binding protein, (CaBP) which eventually lead to increased calcium and phosphorus absorption.

In the chick, intestinal CaBP correlates well with calcium absorbability, and blood CaBP correlates well to intestinal CaBP (Bar et al. 1979). Furthermore, intestinal CaBP in the turkey was found to be identical to that of the chick (Bar et al. 1978).

Some of the metabolites mentioned above may be readily assayed and, thus serve as important tools in the diagnosis of the disorders.

LABORATORY PROCEDURES

Plasma calcium has been determined by an automatic titration with EGTA (Calcette, Precision Systems). For determination of plasma inorganic phosphorus the plasma was treated with trichloroacetic acid and phosphorus was determined in the filtrate by a modification of the method of Gomori (1942) for the use with the Autoanalyze (Technicon).

Bone ash was measured by obtaining the weight of the bone after drying at 105C and ashing at 600C.

For assay of intestinal CaBP, the duodenum was homogenized in Tris buffer and the supernatant was subjected to a radioimmunoassay using ^{125}I-labeled CaBP and a specific antiserum. A similar assay was used for plasma CaBP. Here, however, a greater dilution of the antiserum was practiced, in order to increase sensitivity (Bar et al 1979; Bar and Hurwitz, 1979).

$25(OH)_2D_3$ and $24,25(OH)_2D_3$ were determined by a binding assay, after separation by high performance liquid chromatography, using the ^3H-labeled compound and rat-serum binding-proteins (BioRad Laboratories). $1,25(OH)_2D_3$ was assayed using the receptor assay modified from Bishop et al (1980) with specific receptors isolated from the intestinal mucosa of vitamin D-deficient chickens according to Colston and Feldman (1980).

For the assay of kidney $25(OH)D_3$ hydroxylases, ^3H-labeled $25(OH)_2D_3$ was incubated, in the presence of various cofactors, with kidney homogenates (Montecuccoli et al, 1977). The produced metabolites were then separated by high-performance liquid chromatography and counted in a liquid scintillation spectrometer.

MINERAL METABOLISM IN THE CHICKEN AND TURKEY: A COMPARISON

Most of the information of mineral metabolism in birds is derived from observations made on the chicken. Although no detailed comparisons were made, turkeys appear to be more sensitive then chicks with regard to leg problems.

In order to draw on information derived from the chicken it appeared of importance to compare some parameters of mineral metabolism in chickens and turkeys (Table 1).

Only small differences exist between the two species with regard to plasma minerals and $25(OH)D_3$, although plasma calcium always tended to be slightly higher and $25(OH)D_3$ slightly lower in the turkey than in the chick. Percentage of bone ash in the young turkey has always been above 40% but in the young chick, bone ash is considerably lower, 35-38%.

When the birds are fed with calcium and phosphate at the dietary requirement level, the percentages of calcium and phosphorus absorption are similar in both species. However, calcium and phosphorus absorption per unit of body weight is higher in the turkey. In addition, parameters which are usually increased during calcium deficiency such as the kidney 1-hydroxylase, the concentration of $1,25(OH)_2D_3$ and CaBP in plasma and that of CaBP in the intestine are considerably higher in the turkey. It is also apparent that the absorption of vitamin D is also higher in the turkey. The high intensity of the parameters associated with mineral metabolism appears to suggest a greater sensitivity of the young turkey to its mineral nutrition.

SYMPTOMS OF MINERAL AND VITAMIN D DEFICIENCIES

Important symptoms of calcium, phosphorus and vitamin D deficiency are shown in Table 2. In contrast to the chick, plasma calcium remains almost unchanged during a 3 week vitamin D-depletion period. However a decrease in plasma calcium has been observed in another experiment after 4 weeks on the vitamin D depletion regime. Similarly, plasma calcium in the turkey also resists long periods of calcium deprivation. On the other hand, phosphorus deficiency is characterized by hypercalcemia. Plasma inorganic phosphorus hardly changes during calcium deficiency, but is lowered during phosphorus or vitamin D deficiencies.

Percentage of bone ash is obviously reduced with all three deficiencies. In contrast to the chick, intestinal CaBP hardly changes during calcium or phosphorus deficiency. This may be due to the fact that the normal concentration of this protein in the turkey is relatively high (Table 1). During D-deficiency, the vitamin D-dependent CaBP declines until it virtually disappears. The activity of the enzyme 25 hydroxycholecalciferol-1-hydroxylase, responsible for synthesis of $1,25(OH)_2D_3$, is considerably increased during vitamin D and calcium deficiencies, and only slightly increased during phosphorus deficiency. The 24-hydroxylase activity decreases during vitamin D and calcium deficiencies.

The occurrence of field rickets is usually complicated by other manifestations of disease so that the symptoms may not be as clear as when produced under laboratory conditions. Therefore, more than a single test appears to be necessary for a good diagnosis. Clearly plasma calcium can give only some suggestions as for the source of deficiency. Intestinal CaBP can differentiate between vitamin D deficiency on the one hand, and calcium and phosphorus deficiencies, on the other. Plasma phosphate, plasma calcium and the activity of the 1-hydroxylase enzyme may be used to differentiate between calcium and phosphorus deficiencies.

ETIOLOGY OF FIELD RICKETS

1. Errors in the mineral supplementation on the diet. In Israel this is a source of some of the problems encountered in the field. A relatively common occurrence is extra-supplementation with sources of calcium. This may happen when a layers diet is fed to the young bird. In this case, the symptoms are similar to phosphate deficiency.

2. Omission of vitamin D supplementation. This is relatively rare since vitamin D is usually contained in vitamin premixes.

3. Low phosphorus availability. The cost of the phosphate supplements contributes heavily to that of the diet. Therefore, the interest of the feed manufacturer and also the grower, is to minimize the concentration of the phosphorus supplement on the diet. However, turkeys were shown to be sensitive to problems of phosphorus availability not encountered in chicks. Years ago it was shown that the availability of phosphorus from non-hydrated dicalcium phosphate for turkeys is considerably lower than that of hydrated dicalcium phosphate, although it was fairly available for the chicken. Our studies also show that monocalcium phosphate is a considerably better source of phosphorus than dicalcium phosphate for the turkey, whereas the advantage of mono over the di calcium phosphate is lower for the chick.

4. Defects in the metabolism of vitamin D. In examining field cases of rickets in turkeys (Hurwitz et al. 1973) we observed typical vitamin D-deficiency symptoms, including an almost complete disappearance of intestinal CaBP. Chemical analysis indicated normal concentrations of calcium and phosphorus in the diet and it was ascertained that the diet contained vitamin D. Injection of high doses of vitamin D was without any therapeutic effects. We postulated, at that time, the presence of a pathogen which interfered with the normal vitamin D metabolism or its action in the largest organs. Similar observations have been made since then. However, the primary cause(s) of this condition has not been identified.

THERAPY

We have just completed one study on the treatment of vitamin D-deficient rickets, and one on treating calcium or phosphorus deficient turkeys is now in progress (Bar, A., Rosenberg, J. and Hurwitz, S., unpublished). Young fast-growing turkeys were raised in a windowless room and were made rachitic by feeding a diet devoid of vitamin D sources, for three weeks. The turkeys were then fed either a regular diet, based on NRC (1977) recommendations and containing 50 ug/kg of vitamin D_3 (2000 IU/kg), or a calcium and phosphorus enriched diet. Each of the diets was given without any additional vitamin D sources, or fortified with additional vitamin D_3, with 1-alfa-hydroxycholecalciferol* or with 25-hydroxycholecalciferol. A remarkable recovery from the deficiency symptoms occurred as early as three days after the change of diets. Additional dietary minerals, or fortification with vitamin D_3 or vitamin D_3 derivative were of no additional advantage. The results of treatment of calcium and/or phosphorus deficient turkeys are not available at the present.

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*Synthetic derivative of vitamin D_3; When given to animals the steps of 1-hydroxylation in the kidney is bypassed.

Of special interest are results of treatment of field rickets diagnosed as the vitamin D-deficient-like (Hurwitz et al, 1973). Unfortunately, field conditions usually do not allow for detailed studies. In one farm, however, we were able to collect some data which could be used for a meaningful comparison. Following an unknown duration of a widespread leg weakness in the flock, accompanied by completely inhibited growth and high mortality, the case was brought to our attention when the birds were 5½ weeks of age. In the farm, some of the birds were killed for analysis, and some 300, severely rachitic chicks were divided into three groups. One served as control and was fed the same diet with no vitamin D treatment, another received 200 µg/kg of 25(OH)D_3 per kg of diet, and a third received 20 µg per kg diet of 1-alfa-hydroxy vitamin D_3. All birds received treatment with various antibiotics. The results of this study are shown in Table 3. The initial value of CaBP was very low, and plasma calcium was slightly lower than normal but the percentage ash was considerably lower than the range of 45-46%, typical of this age group. During 8 days after initiation of the treatment, all vitamin D untreated birds died. The results given for the untreated birds are from other surviving birds. In the latter and in the vitamin D treated groups, CaBP was normal. Remarkably, only 20% of the treated groups died, with the surviving birds exhibiting a striking improvement in the bone calcification. Similar results were also obtained in two other farms. These results suggest the possible usefullness of hydroxylated vitamin D derivatives in treating field rickets.

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Table 1. Parameters of mineral and vitamin D metabolism in the growing chick and turkey

Parameter	Chick	Turkey	Reference
Plasma Ca, mg/dl	9.8	10.8	Bar et al., 1978
Plasma P_i, mg/dl	6.5	7.8	Bar et al., 1978
Plasma 25(OH)D_3, ng/ml	16.2	14.0	Unpublished
Plasma 24,25$(OH)_2D_3$, ng/ml	1.4	-	Unpublished
Plasma 1,25$(OH)_2D_3$, ng/ml	0.14	-	Unpublished
Plasma CaBP, ng/ml	29	131	Unpublished
Bone ash, %	37.6	40.8	Bar et al., 1978
Intestinal CaBP, mg/g	1.1	2.3	Bar et al., 1978
Calcium absorption, %	40	40	Hurwitz et al., 1979
Phosphorus absorption, %	40	50	Hurwitz et al., 1979
Vitamin D absorption, %	66.5	83.6	Bar et al., 1980
Kidney 1-hydroxylase*	3.9	6.1	Bar et al., 1978
Kidney 24-hydroxylase*	19.6	11.1	Bar et al., 1978

* pmole/g/15 min.

Table 2. Characteristics of mineral and vitamin D deficiencies in the young turkey*

Treatment	Control	Low Ca	Low P	D-deficient
Dietary Ca, %	1.42	0.34	1.36	1.45
Dietary P, %	0.80	0.81	0.41	0.85
Body weight, g	393 ± 12	391 ± 10	337 ± 11	210 ± 7
Plasma Ca, mg/dl	10.9 ± 0.3	10.2 ± 0.2	15.1 ± 0.3	10.8 ± 0.7
Plasma P_i, mg/dl	7.3 ± 0.4	7.8 ± 0.7	2.5 ± 0.3	2.8 ± 0.4
Bone ash, %	42.5 ± 0.2	37.4 ± 0.6	32.3 ± 1.0	25.3 ± 0.8
Intestinal CaBP, mg/g	2.1 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	0
Kidney 1-hydroxylase**	2.0	14.7	3.9	47.5
Kidney 24-hydroxylase**	16.1	4.3	19.1	4.2

* The controls, low Ca and the low P birds were 20 days old and had received the experimental diets for 7 days. The D-deficient turkeys were 17 days old and received the D-deficient diet since day-old.

** pmole/g/15 min.

Table 3. Treatment of field rickets in turkeys with hydroxylated vitamin D derivatives

Date, treatment	Mortality %	Plasma Ca mg/dl	Bone ash %	CaBP, intestine mg/g
May 5	-	9.8±0.7	37.2±0.6	0.25±0.08
May 21, untreated	100	10.9±0.8	37.3±0.8	2.11±0.16
May 21, $25(OH)D_3$	20	10.2±0.1	41.3±0.2	2.08±0.18
May 25, $1\alpha(OH)D_3$	20	10.0±0.1	46.9±1.0	2.21±0.11

At May 5, the birds were 5½ weeks old. All 25 birds allocated to the untreated group (control) died during the observation period. The results given for the untreated birds were obtained from other birds in the same flock, not as severely affected. The treatments were 200 µg/kg diet of $25(OH)D_3$ and 20 ug/kg of $1\alpha(OH)D_3$, respectively.

INFLUENCE OF PREBREEDER LIGHTING (SOURCE AND INTENSITY) AND NUTRITIONAL SUPPLEMENTATION ON THE REPRODUCTIVE PERFORMANCE OF TURKEY HENS

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Previous studies at the University of Minnesota have examined the influence of environmental lighting and nutrition on turkey hens during the prebreeding period in relation to subsequent reproductive performance. In experiments reported by El Halawani et al. (1975, 1978) the use of low intensity incandescent light (e.g. 1-30 lux) during rearing has adversely affected egg production. At the 1 lux intensity, egg production was low and decreased more rapidly as the season progressed compared to control hens. Hens reared at intensity of 30 lux averaged slightly less (5.8%) in egg production than hens reared under 180 lux in the two studies. The greater egg production seen with hens reared at 180 lux suggests that the light intensity requirement during development is between 30-180 lux.

To meet the requirement for high intensity light during the growing and holding period and at the same time avoid the additional energy cost, two possibilities may be considered. One is to determine whether a shorter time period at the higher intensity may be adequate. The other is to use light sources which are more energy efficient than incandescent lamps, e.g. fluorescent, high sodium vapor lamps. Both of these possibilities have been examined.

The prebreeder nutrition studies (Waibel et al., 1981) have previously centered on the use of restricted feeding, and of low energy and low protein type diets. In the present study some feed additives which could potentially effect reproductive performance were incorporated into prebreeder and breeder diets. The selected feed additives were vitamin E, ethoxyquin, chromium, and cement dust. Vitamin E has been reported to improve the immune response to bacterial infection and improve tissue stability in poultry. Ethoxyquin has been shown to improve hatchability of chicken eggs and increase long term productivity. Chromium has been shown under some conditions to improve egg quality and is suspected to be an unidentified factor in fermentation by-products. Cement dust has been suggested to contain unidentified trace minerals with growth promoting activity in cattle.

In the two studies reported herein the objectives were to study the influence of light source and intensity during rearing and to study the effect of nutritional additives in prebreeder and breeder diets on reproductive performance.

METHODS

Large White female line hens of the Nicholas strain were used in both studies. The poults (day of age) were distributed randomly into floor pens in the four rooms of the environmental building. At 30 weeks of age (Experiment 1) or 31 weeks (Experiment 2) the hens were moved into floor pens of the breeder pole building. The hens were light stimulated with incandescent lamps (15 hours light daily) and kept at 50 lux intensity during the breeding season. The hens were inseminated with pooled semen of Nicholas male line toms every

10 days. During the production phase, egg production was summarized at 4 week intervals. Fertility and hatchability were determined from one week collection of eggs once every 4 week period.

In Experiment 1, the nutritional treatments were fed starting at day of age and continuing through the breeding cycle. The dietary treatments were formed by the supplementation of various additives to the growing, holding and breeder diet. The control treatment was a simplified corn-soybean meal type diet. Individual additions to the control diet were formed by chromium (10 ppm), cement dust (3%), vitamin E (300 I.U./kg), and ethoxyquin (.015%). The sixth diet which is identified as the complex diet contained all of the above additives plus some ingredients with unidentified factor activity (fish solubles, fermentation product, zinc-methionine, and antibiotic).

The lighting treatments were begun at eight weeks of age. Prior to this time, all birds were kept at 5 lux of incandescent light. The lighting treatments were: incandescent light continuing at 5 lux to 30 weeks of age; incandescent light at 180 lux for 8-30 weeks; high pressure sodium vapor at 1,200 lux for 8-30 weeks; incandescent light continuing at 5 lux and changing to 180 lux at 18 weeks of age. Two groups of birds were also moved to range pens at eight weeks of age where light intensity was high and variable. Measurements of sunlight gave intensities of approximately 30,000 lux on a cloudy day and 106,000 lux on a sunny day.

In Experiment 2, all hens were fed the same growing and holding diets until the breeding period when half the pens were fed the simplified corn-soybean diet and the other half were fed the complex diet. For the lighting treatments (8-31 weeks of age), the same experimental plan was used as in Experiment 1, excepting that the treatments previously given 5 lux intensity were changed to 30 lux.

RESULTS AND DISCUSSION

The results of Experiment 1 are shown in Table 1 and are summarized by means for the main effects of diet and light treatment. Diet did not significantly affect egg production, fertility or hatchability for the 24 week production period. Hens fed the complex diet did have numerically better egg production than control fed hens (49.4 vs. 54.2%), but showed no advantages in fertility or hatchability.

Confinement light treatment significantly ($P < .05$) affected egg production but not fertility or hatchability. Egg production was significantly lower for hens reared at low light intensity (5 lux or 5 changing to 180 lux) when compared to hens reared at 180 lux. Hens reared under sodium lighting and range environments had similar egg production rates which were slightly less than 180 lux treated hens.

The adverse effect of low light intensity treatment on egg production appeared more evident as the laying cycle advanced. Hens from the low intensity light treatments decreased their egg production at a more rapid rate after 12-16 weeks of production. This rapid decrease in egg production was also seen for hens reared under 5 lux intensity changing to 180 lux at 18 weeks of age.

The results of Experiment 2 are summarized in Table 2. Diet did not significantly affect egg production, but hens fed the complex diet again showed a numeric increase in egg production (+1.5%) over the control hens. Hens fed the complex diet had significantly ($P < .05$) better fertility by 4.5%. The fertility effect was particularly evident in the latter part (16-24 weeks) of the laying season. Whether this was a true change in fertility or early embryonic mortality is unknown because fertility was determined by candling and not by examination of the germ cell in the egg.

Light treatment did not significantly affect egg production although hens reared at 30 lux had a slightly lower level of egg production, especially when compared to the range birds and sodium vapor light treatment.

In summary, feeding the complex diet to turkey breeder hens did not significantly affect egg production, although the hens fed the complex diet did show a numeric increase in egg production averaging 3% better than the control hens fed the simplified corn-soy diet in both studies. In one experiment, the complex diet significantly ($P < .05$) improved fertility. Light intensity significantly affected egg production in Experiment 1 but not in Experiment 2. The results in Experiment 1 indicated that rearing hens with 5 lux intensity adversely affected egg production and could not be counteracted by switching to high intensity light (180 lux) at an older age (18 weeks of age). In both experiments, hens reared under sodium vapor lighting and on-range had egg production rates similar to hens reared at 180 lux intensity. Birds reared under 30 lux for 8-31 weeks in Experiment 2 had slightly lower egg production than the controls, suggesting this light intensity is also inadequate.

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Table 1. Effect of light and nutrition treatments on egg production, fertility, hatchability and body weight over the 24-week production period, Experiment 1.

Variable	Egg Production %	Fertility %	Hatchability of fertile eggs, %	Body weight (kg) Age (weeks) 33	57
Dietary treatment[a]					
Control	49.4	84.8	86.5	9.1	8.8[b]
Chromium, 10 ppm	53.9	78.9	84.2	9.1	8.8
Cement dust, 3%	52.4	81.3	87.1	8.9	8.6
Vitamin E, 300 I.U./kg	52.4	81.1	84.7	9.0	9.0
Ethoxyquin, .015%	48.2	80.9	86.0	9.0	8.8
Complex	54.2	82.4	86.4	8.9	8.5
Light treatment					
Source — Intensity (lux)					
Age: 8-18 / 18-30 wks					
Incandescent 5 / 5	49.5[c]	80.0	85.0	8.9	8.7
Incandescent 180 / 180	56.5	81.8	84.7	9.0	8.8
Sodium vapor 1200 / 1200	53.6	81.6	87.4	9.0	8.7
Incandescent 5 / 180	47.4	82.9	86.0	9.1	8.8
Range -- / --	53.2	85.5	85.1	9.9	9.2

[a]See text for description.

[b]Diet significantly ($P < .05$) affected body weight (57 weeks).

[c]Confinement light treatment signficantly ($P < .05$) affected egg production.

Table 2. Effect of light and nutrition treatments on egg production, fertility, hatchability and body weight over the 24-week production period, Experiment 2.

Variable	Egg Production %	Fertility %	Hatchability of fertile eggs, %	Body weight (kg) Age (weeks) 34	58
Dietary treatment[a]					
Control	54.0	70.5[b]	75.2	9.0	8.6
Complex	55.5	75.0	77.3	9.2	8.6
Light treatment					
Source — Intensity (lux)					
Age: 8-18 / 18-31 wks					
Incandescent 30 / 30	52.8	69.9	76.1	9.2	8.7
Incandescent 180 / 180	54.6	74.1	73.4	9.2	8.6
Sodium vapor 1200 / 1200	56.9	73.4	77.8	9.0	8.4
Incandescent 30 / 180	53.1	69.8	73.7	9.2	8.6
Range -- / --	56.3	76.4	80.5	9.3	8.6

[a]See text for description.

[b]Diet significantly ($P < .01$) affected fertility.

SOLAR HEATING FOR MINNESOTA TURKEY GROWERS? YES, IF!

by

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Thermal performance simulations of turkey ventilation systems indicate that solar systems can provide a portion of the supplemental heating requirements. The solar energy supplied, QSOLS, may be compared to the capital and operating costs of the associated collector/storage system, to determine economic viability of the system.

The economic model most applicable involves valuation of all projects costs during the study period, or life cycle, of the project. Using life cost analysis procedures, all future costs are discounted to present values and the comparison may be made between alternatives on the basis of minimization of life cycle costs. The method of valuation of future costs developed by Brandemuehl (1978) is used as the economic basis of the "f-chart" collector sizing method and may readily be applied to the results of simulation studies.

The application of life cycle costing allows for calculation of two related, but differently interpreted, quantities. These two quantities are life cycle savings (SAV), realized by the solar system and the internal rate of return or return on investment (ROR). Life cycle savings, (SAV) is typically applied in the design phase, by generating SAV as a function of collector area and selecting an optimum collector area, which corresponds to a maximum value of SAV. Rate of return, (ROR), calculations are typically performed to indicate economic viability to prospective users of the alternative systems.

LIFE CYCLE SAVINGS

Life cycle savings (SAV), due to the use of the solar system is given in equation (1) as the difference between the life cycle costs of a conventional system (CONV) and the solar system (SOLC).

$$SAV = CONVC - SOLC \qquad (1)$$

Equation (1) has been expanded by Brandemuehl (1978) to include all components of life cycle costs. With E = e - k - ek, the savings equation becomes:

$$
\begin{aligned}
SAV = {} & (1 - Z * TB)\ (CFC - CFS)\ (QLOAD)\ f(NE, E, d) \\
& + (1 - Z * TB)\ (CFS)\ (QSOLS)\ f(NE, E, d) \\
& - (D)\ (INV) \\
& - (1 - D)\ \left[\frac{f(N1, 0, d)}{f(NL, 0, i)}\right]\ (INV) \\
& - (1 - Z * TB)\ (M)\ f(NE, g, d)\ (INV) \\
& + (1 - D)\ (TB)\ \left[\frac{f(N1, 0, d)}{f(ML, 0, i)} + f(N1, i, d)\ \left(i - \frac{1}{f(NL, 0, i)}\right)\right]\ (INV) \\
& - t(1 - TB)\ (v)\ f(NE, g, d)\ (INV) \\
& + Z\ (TB)\ (U)\ (INV) \\
& + \left[\frac{G}{(1 + d)^{NE}}\right]\ (INV) \\
& + (TCf\ (1, 0, d)
\end{aligned}
\tag{2}
$$

The factor f (a, b, c) is a discount-inflation factor and is given in analytical form in (3).

$$
\begin{aligned}
f\ (a, b, c) &= \frac{1}{(c - b)}\ \left[1 - \left(\frac{1 + b}{1 + c}\right)^{a}\right];\ b \neq c \\
&= \frac{1}{(1 + b)} \qquad ;\ b = c
\end{aligned}
\tag{3}
$$

The discount-inflation factor, f (a, b, c), given in (3) is a factor, which, when multiplied by the end of first period cost or annuity value will give the present worth of the cost or annuity (which is inflated at rate b and discounted at rate c over a periods). With b = 0, this factor is reduced to the familiar series present worth factor.

If the assumption is made that the costs of fuel are equal (CF = CFC = CFS), then equation (2) may be collapsed to the simpler form of (4).

$$
SAV = P1\ (CF)\ (QSOLS) - P2(INV) + (TC)f(1, 0, d) \tag{4}
$$

where

$$
P1 = (1 - Z * TB)\ f(NE, E, d) \tag{5}
$$

$$P2 = D + (1 - D)\left[\frac{f(NL, 0, d)}{f(NL, 0, i)}\right] + (1 - Z * TB)(M) f(NE, g, d) - (1 - D)(TB)\left[\frac{f(NL, 0, d)}{f(NL, 0, i)} + f(NL, i, d)\left(i - \frac{1}{f(NL, 0, i)}\right)\right] - t(1 - TB)(V) f(NE, g, d) - (Z * TB)(U) - \left[\frac{G}{(1 + d)^{NE}}\right] \quad (6)$$

In equation (6), the first and second terms of P2 are due to the down payment and loan amortization, the third is due to miscellaneous costs, the fourth is due to income tax deductions, the fifth is due to property taxes and the sixth and seventh are due to depreciation and salvage value, respectively.

- In general, the terms are written to include the discount rate, d, which is considered a market discount rate (includes the effects of inflation) and represents the opportunity cost of money.

- The miscellaneous costs include all maintenance, insurance and parasitic power costs, which are assumed to inflate at the general inflation rate.

- The income tax deductions are due to interest and property tax payments which are deductible from federal and some state taxes, in the case of owner-occupied residences. Most tax laws also allow deductions of operating expenses for commercial operations.

- The property tax model assumes that the real value of the solar system remains fixed throughout the lifetime. That is, the market value, which is assumed equal to the initial investment, increases with the general inflation rate, g. Care must be exercised to be consistent between the property tax model and the salvage value because if property taxes are in effect, the salvage value (G) should necessarily be equal to the market value in the year of salvage.

- The function, U, which is consistent with the economics used in the development, should be taken as given in equation (7) for straight-line depreciation. U functions for double declining balance and sum of years digits depreciation methods are given in Duffie (1980).

$$U = f(ND, 0, d)/ND \quad (7)$$

- The last term in the savings equation (4) represents the current tax credit, which is available for qualifed alternative energy system investments. For the credit to be available, a tax liability equal to TC must be generated by

the investor. The tax credit, TC, is discounted by one year to indicate its availability one year hence in the cash flow. Current tax credits are available according to the following schedule.

Residential/Family Farm

Federal TC = 40% of the first $10,000 of (INV)
Minnesota TC = 20% of the first $10,000 of (INV)

Business/Commercial

Federal TC = 15% of (INV)

RATE OF RETURN

The rate of return (ROR), also known as the Profitability Index (Grant, et al. (1976)), is defined as the discount rate at which the present worth of cash flow is equal to zero. This would suggest that the rate of return, ROR, would satisfy equation (1) with SAV = 0. That is, the rate of return is the discount rate at which the life cycle savings present worth is at a "break-even" condition.

The calculation of ROR is basically an iterative procedure with computations involving the expanded form of (4). The rate of return computed was the discount rate of which SAV = 0 in (4) and was interpolated to the nearest one tenth percentage. The computed rate of return, ROR, may be considered an after tax, internal rate of return.

ECONOMIC PARAMETERS

The calculation of life cycle savings, (SAV) and rate of return (ROR) requires assumptions on thirteen economic parameters. Table 1 summarizes the ranges of the parameters and indicates three comparison cases. The (REF1) case reflects a "current" scenario for assumptions on Minnesota farm economics.

Table 1. Economic Parameter Conditions.

Parameter	Symbol	Range	REF1	Base	Comp.
Salvage	G	0-100%	0	0	0
Degradation	K	0-1.5%	0	0	0
Maintenance	M	1-3%	1	1	1
Property Tax	t	0-4%	0	0	2
General Inflation	g	6-15%	10	7	7
Discount Rate	d	0-15%	14	10	9
Mortgage Interest Rate	i	8-15%	14	10	10
Fuel Escalation	e	6-15%	14	7	11
Down Payment	D	0-100%	15	15	15
Marginal Tax Bracket	TB	20-48%	30	30	38
Study Period	NE	0-30 YR	15	20	30
Loan Life	NL	0-20 YR	7	20	30
Depreciation Life	ND	0-20 YR	7	20	30

While an economic sensitivity analysis indicating the sensitivity of the SAV or ROR functions to the independent parameters of Table 1 is most desirable, it is beyond the scope of discussion here and is left to the reader's investigation. However, there are current "rules" which are plausible to aid in the selection of parameters for farm-use application.

- Minnesota currently does not assess a property tax on solar installations (t = 0). The COMP economics of Table 1 indicate a more "solar optimistic" national situation.

- If the argument that fuel inflation is responsible for driving general inflation is accepted, it is judicious to use a value for fuel inflation which is 3 - 4 percent greater than general inflation.

- Agricultural lending institutions suggest that loans on alternative energy systems might be considered for NL equal to 5 to 7 years, with 15 percent as a down payment.

- The discount rate should logically be equal to the mortgage interest rate for SAV calculations. The discount rate in ROI calculations is a "floating" parameter.

- The method of depreciation used herein is straight-line and the depreciation life is set equal to the loan life as a default value.

- The marginal tax bracket is strictly an individual situation assumption.

ECONOMIC ANALYSIS APPLICATION

The application of life cycle savings and return on investment calculations require knowledge of values for CF, QSOLS and INV.

QSOLS is identified in simulation results at three minimum air flow rates (0.25, 0.30,and 0.35 CFM/lb) for four different collector areas (1800, 3600, 7200 and 14400 sq. ft.) at three different geographic locations (Bismarck, ND, Fresno, CA and Charleston, SC). Typical Meterological Year (TMY) weather data from the National Climatic Center (NOAA) was used, along with the ventilation conditions indicated in Table 2, as input to the computer simulation.

The first year cost of delivered fuel energy, CF, is identified at three levels using \$0.55, \$0.65 and \$0.75/gallon of propane and is calculated on the basis of 80 percent overall combustion efficiency.

The total investment cost, INV, is calculated as the collector gross area times the installed cost of the collector system per unit area. The manufacturer's suggested installed cost of the air collector used in the simulation is presently \$12 - \$18/sq ft, depending upon the size of the collector. It has been suggested in the literature, Kohler (1979), and by experience that similar performance may be obtained from site-built collectors costing \$7/sq ft. The incremental cost of rock bed storage was assumed to be \$3/sq ft of collector area.

TABLE 2. SUMMARY OF CONDITIONS FOR SIMULATION RUNS

TYPE	TURKEY FACILITIES BIRDS #	TEMPERATURE F	AIRFLOW CFM/LB.	COLLECTOR TYPE	COLLECTOR STORAGE	WEATHER STATION
BROODER	5,000 TOMS/ 5,000 HENS	95 ↓5F/WK	0.25	LIQUID	YES	MADISON,WI
			0.30			
			0.35			
BROODER	5,000 TOMS/ 5,000 HENS	95 ↓5F/WK	0.25	AIR	NO	MADISON,WI
			0.30			
			0.35			
GROWER	5,000 TOMS	55	0.25	AIR	NO	MADISON,WI
			0.30			
			0.35			
GROWER	10,000 TOMS	60	0.25	AIR	NO	BISMARCK,ND
			0.30			
			0.35			
GROWER	10,000 TOMS	60	0.25	AIR	YES	BISMARCK,ND
			0.30			
			0.35			
GROWER	10,000 TOMS	65	0.25	AIR	NO	BISMARCK,ND
			0.30			
			0.35			
GROWER	10,000 TOMS	65	0.25	AIR	YES	BISMARCK,ND
			0.30			
			0.35			
BROODER	10,000 HENS	95 ↓5F/WK	0.25	AIR	NO	BISMARCK,ND
			0.30			
			0.35			
BROODER	10,000 HENS	95 ↓5F/WK	0.25	AIR	YES	BISMARCK,ND
			0.30			
			0.35			
BROODER	10,000 TOMS	95 ↓5F/WK	0.25	AIR	NO	BISMARCK,ND
			0.30			
			0.35			
BROODER	10,000 TOMS	95 ↓5F/WK	0.25	AIR	YES	BISMARCK,ND
			0.30			
			0.35			
BROODER	10,000 TOMS	95 ↓5F/WK	0.25	AIR	NO	FRESNO,CA
			0.30			
			0.35			
BROODER	10,000 TOMS	95 ↓5F/WK	0.25	AIR	YES	FRESNO,CA
			0.30			
			0.35			
BROODER	10,000 TOMS	95 ↓5F/WK	0.25	AIR	NO	CHARLESTON,SC
			0.30			
			0.35			
BROODER	10,000 TOMS	95 ↓5F/WK	0.25	AIR	YES	CHARLESTON,SC
			0.30			
			0.35			

LIFE CYCLE SAVINGS

The intended use of the life cycle savings (SAV) is for optimization of the collector area for any given set of economic parameters and heating load requirements. Figure 1 illustrates SAV plotted as a function of collector area for a tom brooder barn using the economic parameter labeled REF1 in Table 1 at a total collector/storage cost of $10/sq ft. It is apparent from Figure 1 that at the 0.35 CFM/lb minimum ventilation rate, the "optimum" collector area, where SAV is maximized, is approximately 3000 sq ft. The optimum areas of 0.30 and 0.25 CFM/lb are approximately 1800 and 1500 square feet, respectively. The optimum areas at the 0.25 and 0.30 rates are less well founded, since the minimum collector area simulated was 1800 sq ft and therefore the nature of the SAV curves less than this area is not well known.

Figure 1 suggests that increasing the ventilation rate increases the "optimum" collector area. It is also apparent that increasing the ventilation rate has positive effect in terms of increasing the absolute level of life cycle savings. The increased level of life cycle savings may be put into proper perspective by examining Figure 2, which shows the effects of ventilation rate on life cycle costs as well as savings. It is obvious that the increased savings due to increasing the ventilation rate cannot be justified, since the life cycle costs are so dramatically increased. The conclusion is that conservation is the first step in considering any alternative energy system.

Figure 3 indicates the effect of adding 0.85 cubic feet of rock storage per square foot of collector at the incremental cost of $3/sq ft. Again, the shape of the curve has been altered, but the actual optimum area is not well defined, since 1800 square feet was the minimum area simulated.

The effects of life cycle savings of the current application of energy tax credits are shown in Figure 4. The marked decrease in SAV indicates the critical role that the tax credits play in the economic assessment. For this reason, it is imperative that any prospective user of solar systems be fully aware of the applicable tax law requirements. The assumption used herein is that the installer can generate a tax liabilty equal to the tax credit available.

RATE OF RETURN

The rate of return calculation is based on a market discount rate, which has not been adjusted for general inflation. The after-tax, internal rate of return calculated may be used as an effective comparator of the relative attractiveness of the investments. Under the conditions stated, an economically logical minimum attractive rate of return might be considered equal to the long term mortgage interest rate.

In the current analysis, the loan interest rate used was 14 percent, which when applied as a minimum ROR in Figure 5 would suggest maximum allowable installed collector/storage system costs of approximately $12, $14, $15.50/sq ft for the ventilation rates of

0.25, 0.30 and 0.35 CFM/lb, respectively. Increasing the ventilation rate to increase the ROR may be argued again by the life cycle cost increase.

Figure 6 indicates the allowable increased cost of adding rock storage to the brooder system. For example, at a minimum ROR at 14%, one can "afford" $10/sq ft for a collector without storage and $12/sq ft for collector with storage.

Figure 7 illustrates the very dramatic effect of the tax credits on the ROR calculations.

Figure 8 compares the relative merits of using solar heating in the two different systems of growing and brooding barns. Figure 9 indicates a relative geographical coparison between the sun belt and a Minnesota type climate. Both Figure 8 and 9 should be viewed cautiously, since the area of 1800 sq ft used is not the "optimum" under the given economic scenario. More data for QSOLS, the solar energy supplied would be required to adequately address the "optimum" sizing at areas less than 1800 sq ft.

The economic sensitivity issue has been purposely avoided, because the analysis requires the assumptions of 13 key parameters. Figure 10 reveals the effect of varying one of these parameters over a "reasonable" range. Namely, the change in ROR shows a marked influence with the assumption on fuel escalation rate. The ultimate economic consideration can only be performed on an individual basis and is drastically affected by each individual's perception of future economic conditions.

CONCLUSIONS

1. The economics of solar heating in turkey ventilation systems are only as good as the "crystal ball" which is used.

2. Simulation provides a reasonable method of estimating solar energy benefits and economic criteria which can be used for optimization of designs.

3. The only economic variable which is "controllable" by the individual is the cost of the collector. It is suggested that farmers consider site-buildings to minimize installed costs.

4. Because of the complexity of the relationship between heat requirements and solar energy utilized, additional field verification of solar energy use is desirable.

5. Simulation programs should be developed and made available to farm groups to allow answering the questions of cost of solar energy utilized.

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APPENDIX

Nomenclature

A	collector area
CF	cost of delivered energy when CFS = CFC
CFC	cost of delivered energy for conventional system
CONVC	life cycle cost of conventional heating system
d	annual market discount rate
D	ratio of down payment to initial investment
e	annual market rate of fuel price inflation
E	defined as e - k - ek
g	annual rate of general inflation
G	ratio of salvage or resale value to initial investment
INV	initial solar heating system investment
K	annual rate of solar system performance degradation
M	ratio of first year miscellaneous costs to initial investment
N	common life cycle analysis lifetime
ND	depreciation lifetime
NE	duration of life cycle cost analysis
NL	loan amortization period
NI	number of annual loan payments which contribute to life cycle cost analysis
P1	factor relating life cycle cost of fuel savings associated expenses to first year fuel savings
P2	factor relating life cycle cost to investment associated expenses to initial investment
QSOLS	annual solar energy utilized in ventilation system
QLOAD	annual ventilation air make up heating load
QAUX	annual auxiliary heating load (= QLOAD - QSOLS)
ROR	after-tax, internal rate of return

SAV	life cycle savings of solar-assisted heating system over conventional heating system
SOLC	life cycle cost of solar assisted heating system
t	property tax rate based on assessed value
TB	effective state and federal income tax rate (marginal tax bracket)
TC	effective state and federal energy tax credit rate
U	ratio of life cycle depreciation cost to initial investment
V	ratio of assessed value in first year to initial investment
Z	commercial/non-commercial flag (= 0 for non-commercial and = 1 for commercial situations)

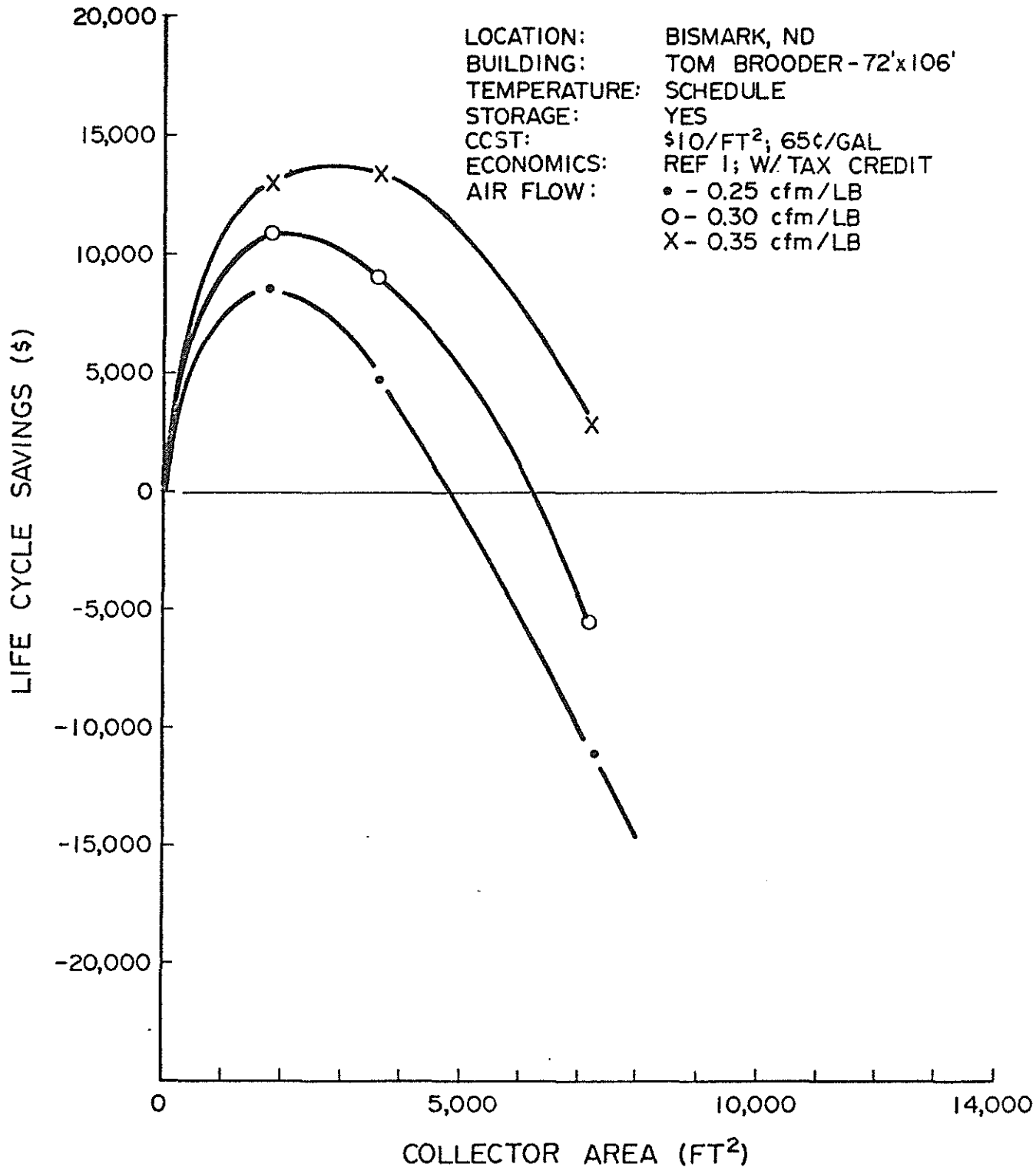

Figure 1. Life cycle savings is determined for three ventilation rates as a function of collector area.

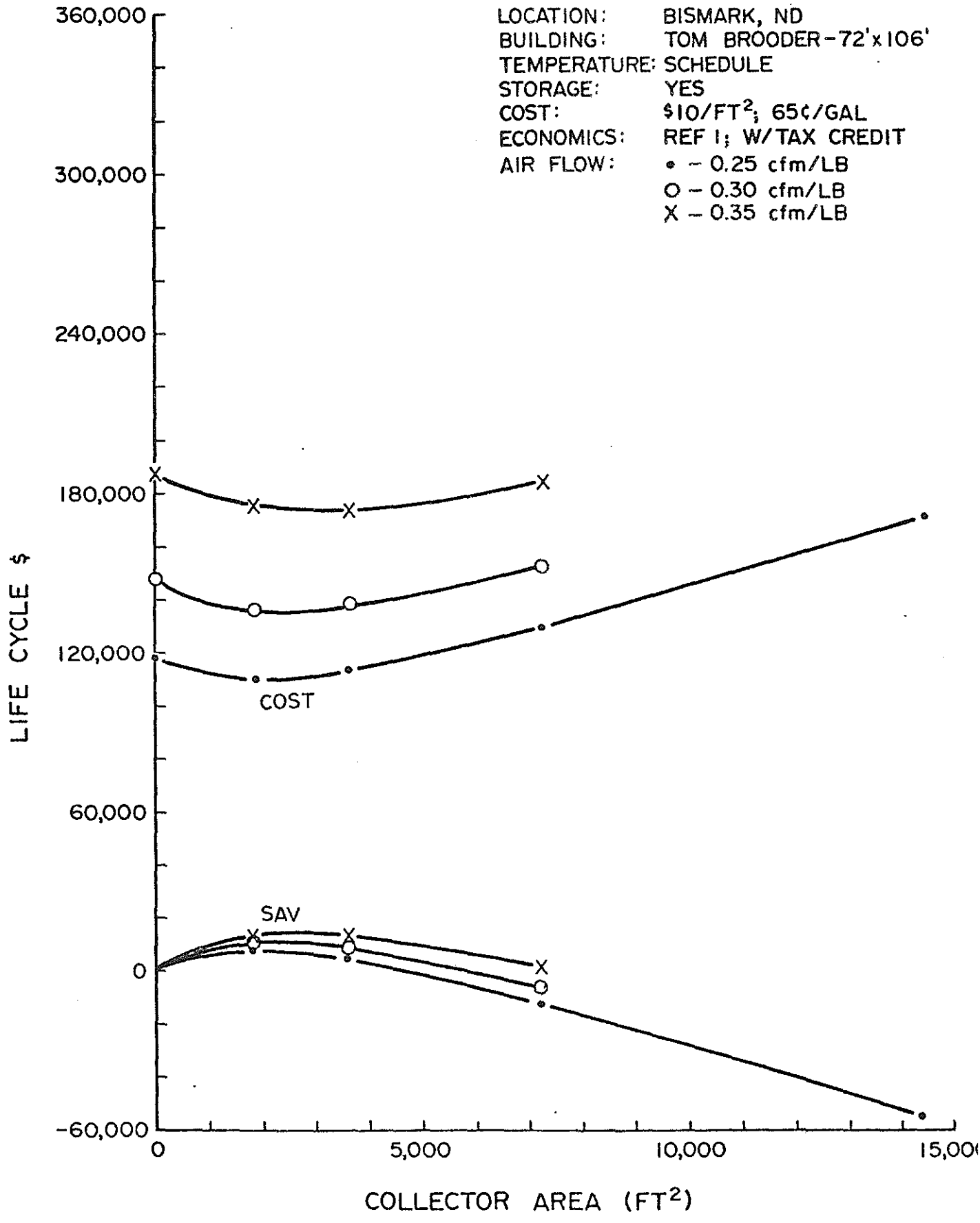

Figure 2. Life cycle costs are slightly affected by collector area but are markedly affected by ventilation rate.

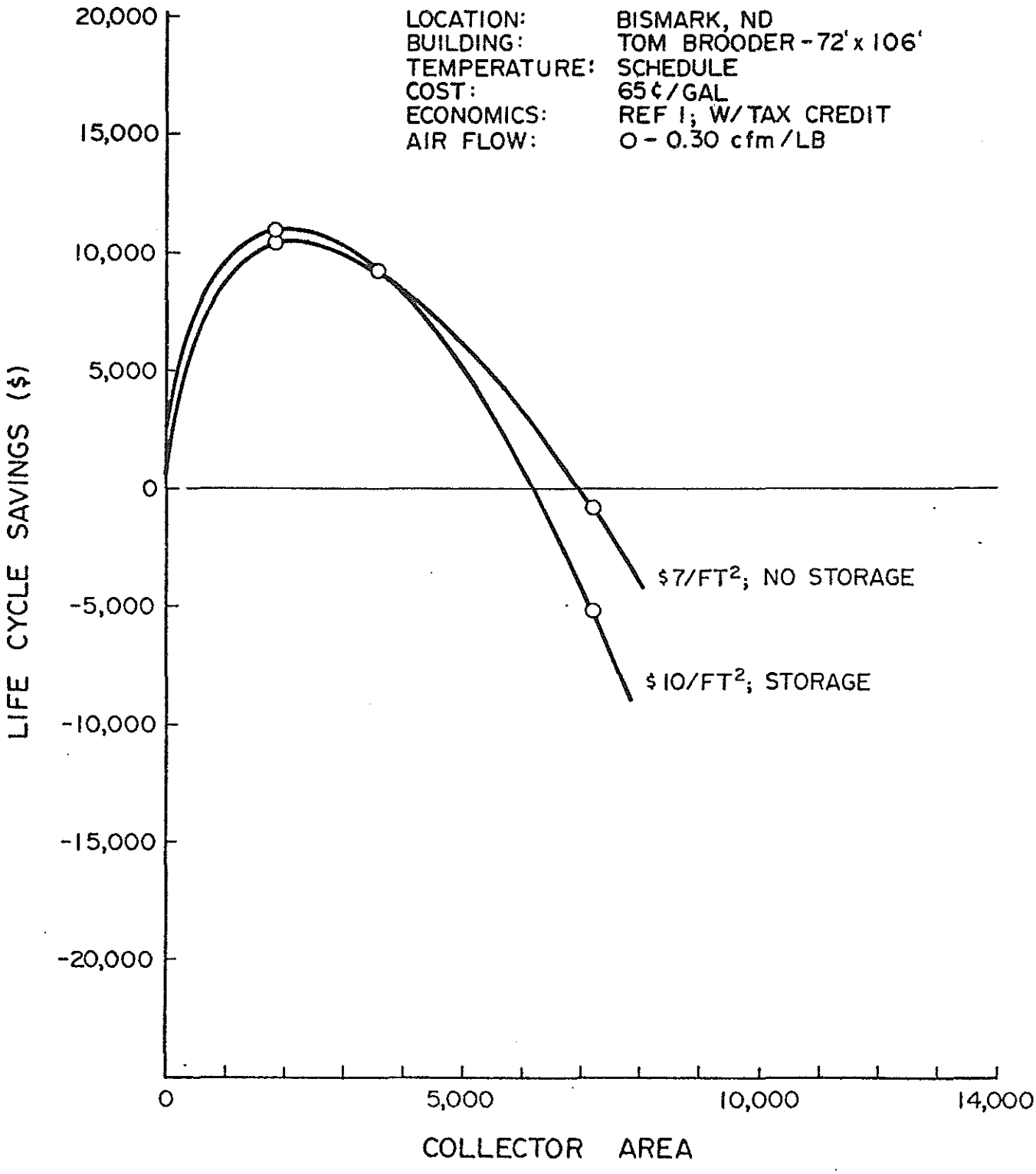

Figure 3. The effect of storage at $3/sq ft is not significant on optimum area.

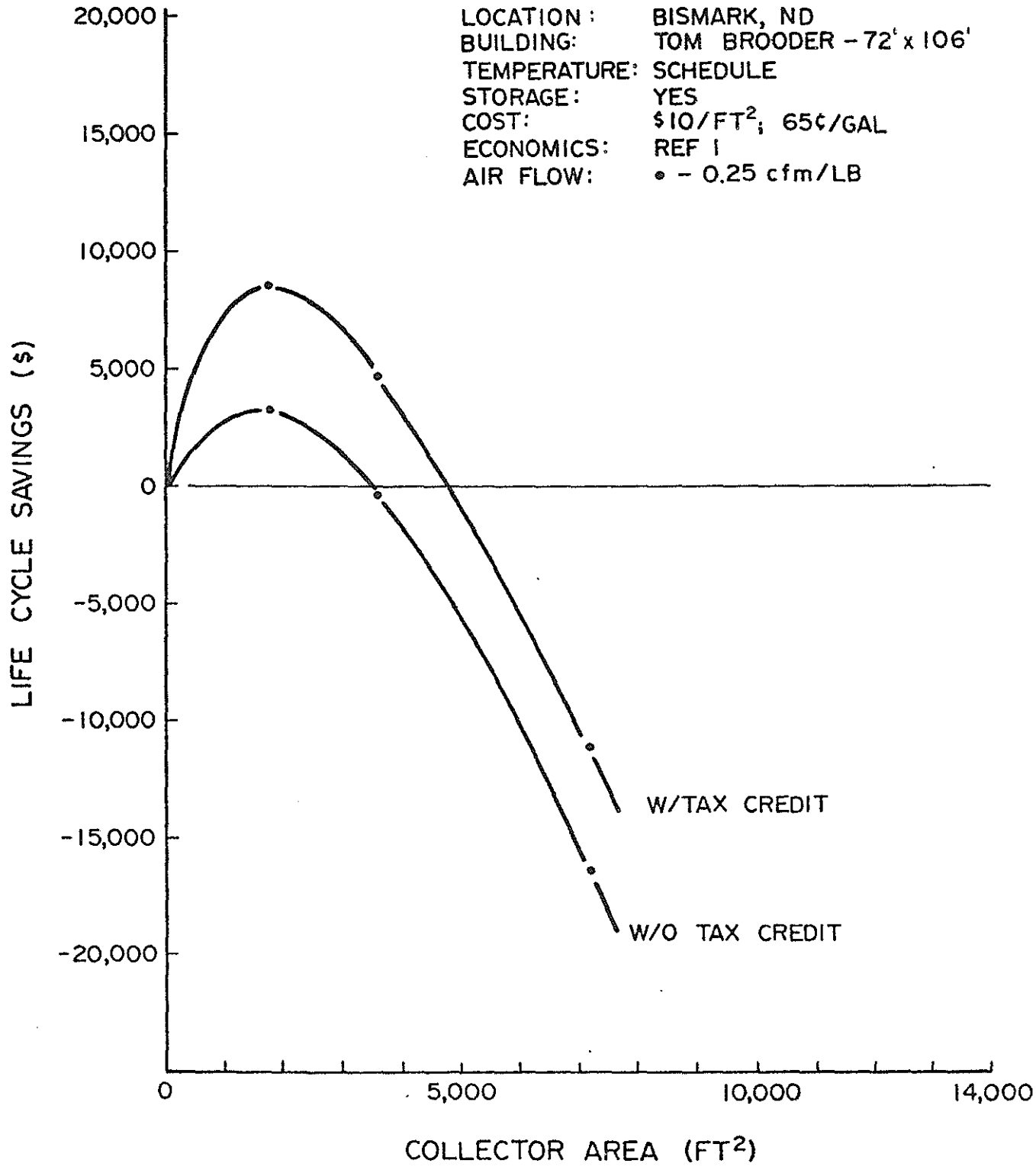

Figure 4. The effect of current tax credits on life cycle savings is significant.

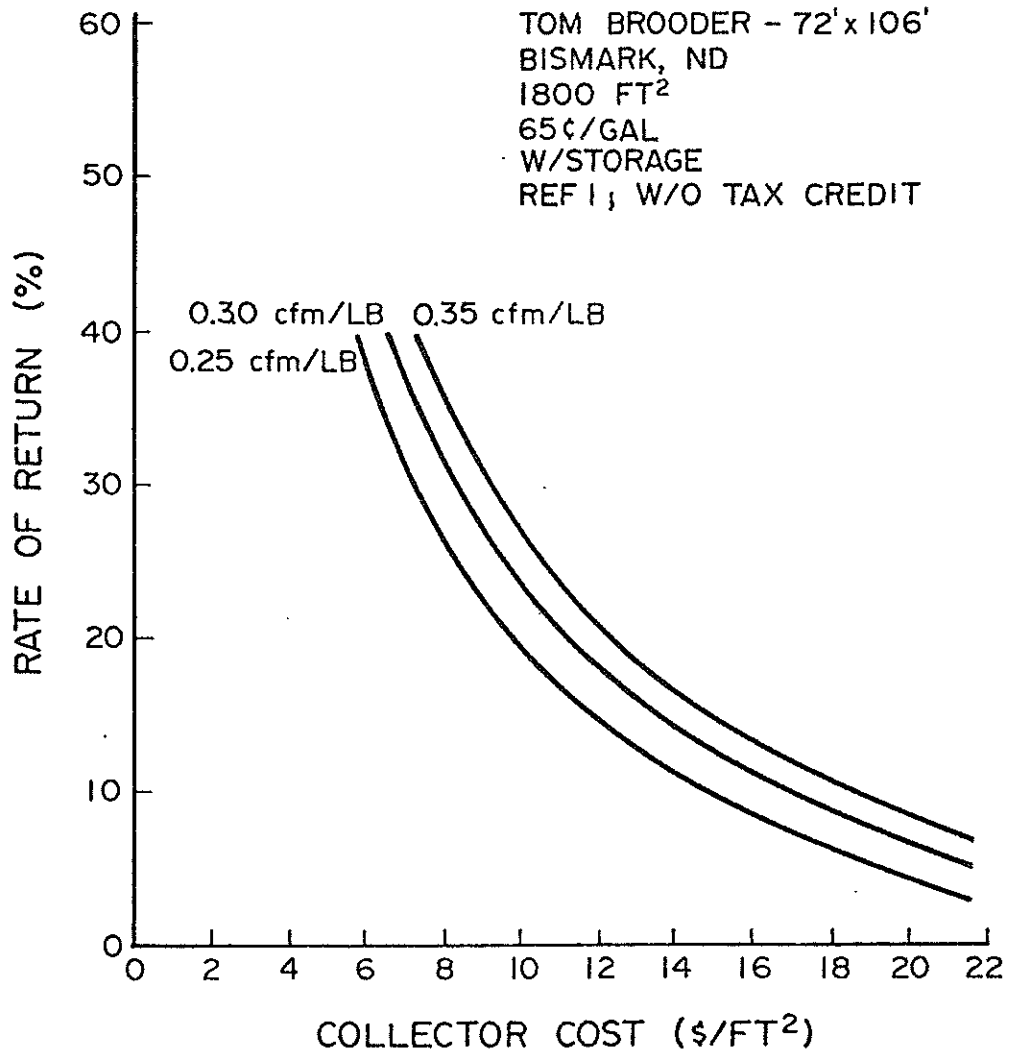

Figure 5. The ventilation rate influences rate of return.

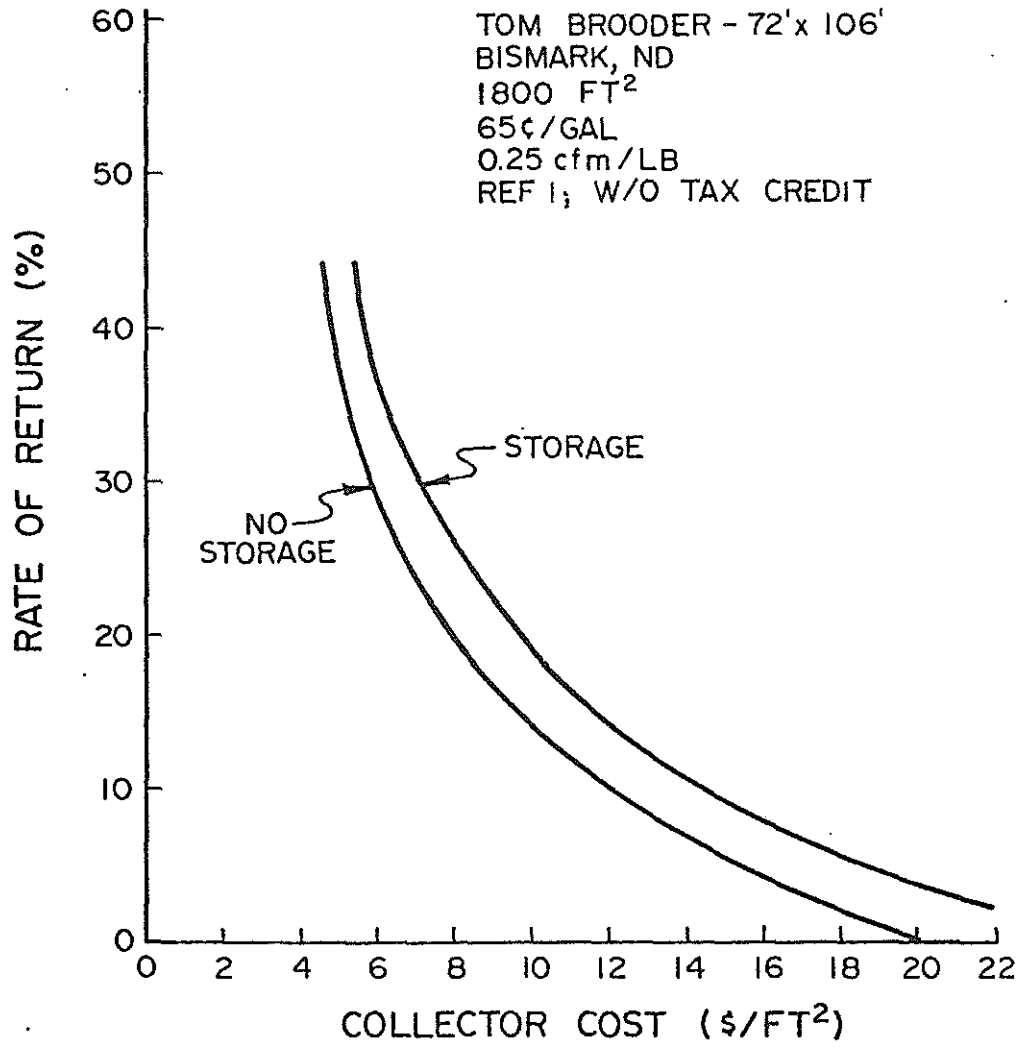

Figure 6. Rate of return may be used to determine allowable incremental storage costs.

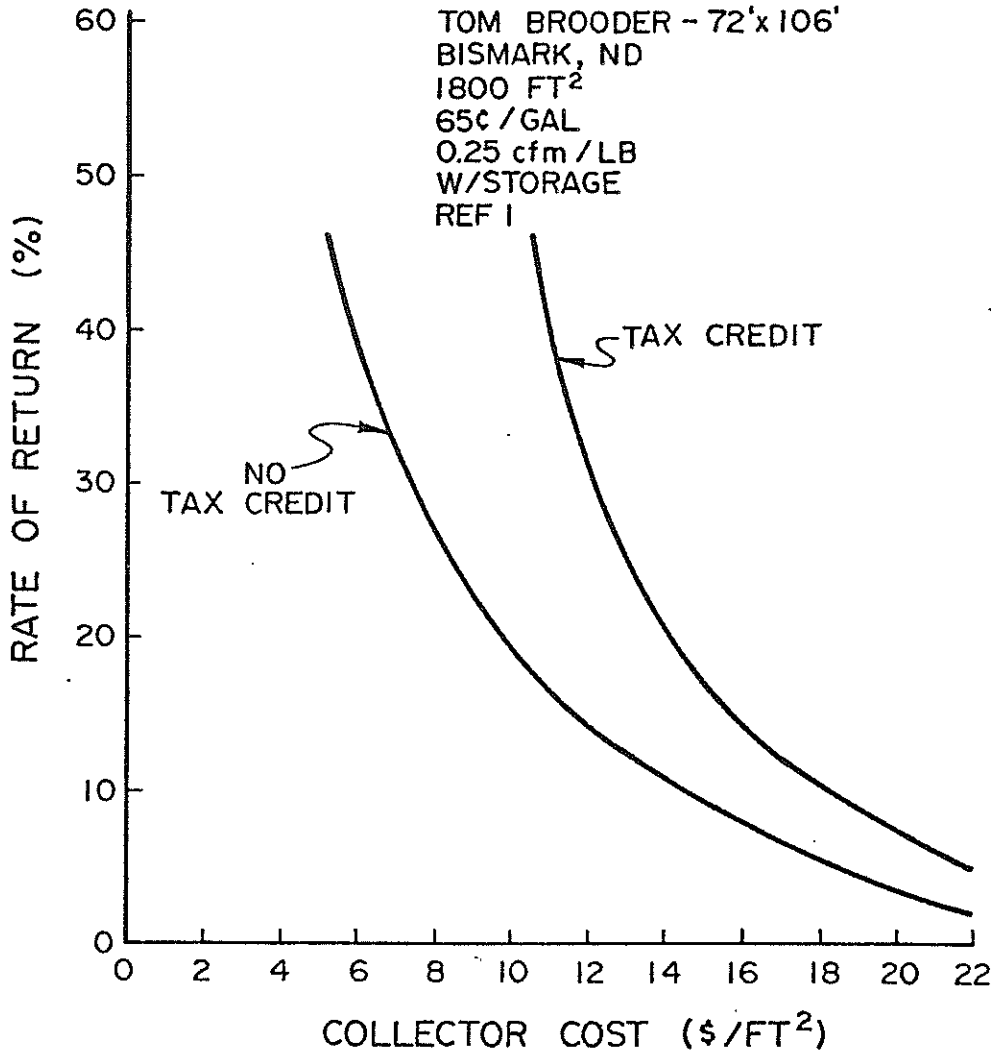

Figure 7. The energy tax credit significantly affects ROR.

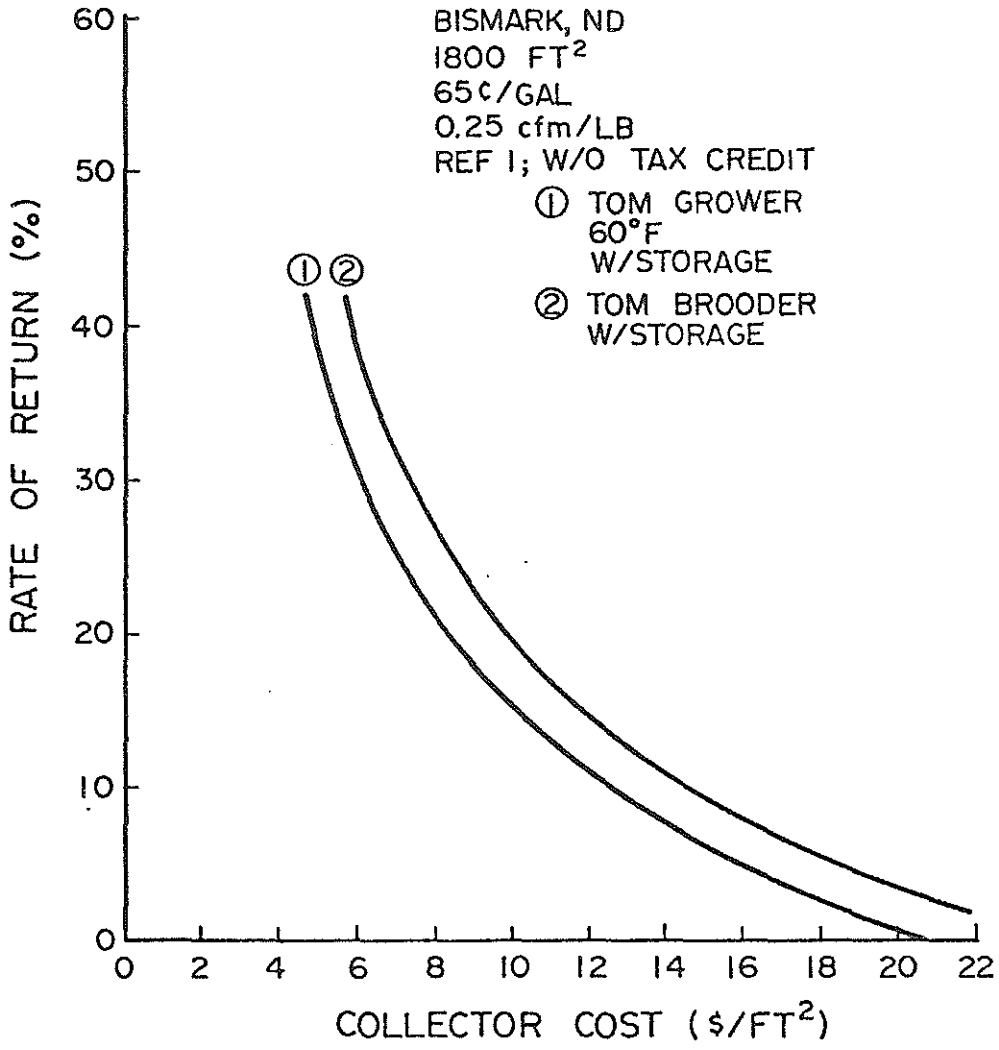

Figure 8. The relative attractive ness of two systems may be suggested by rate of return.

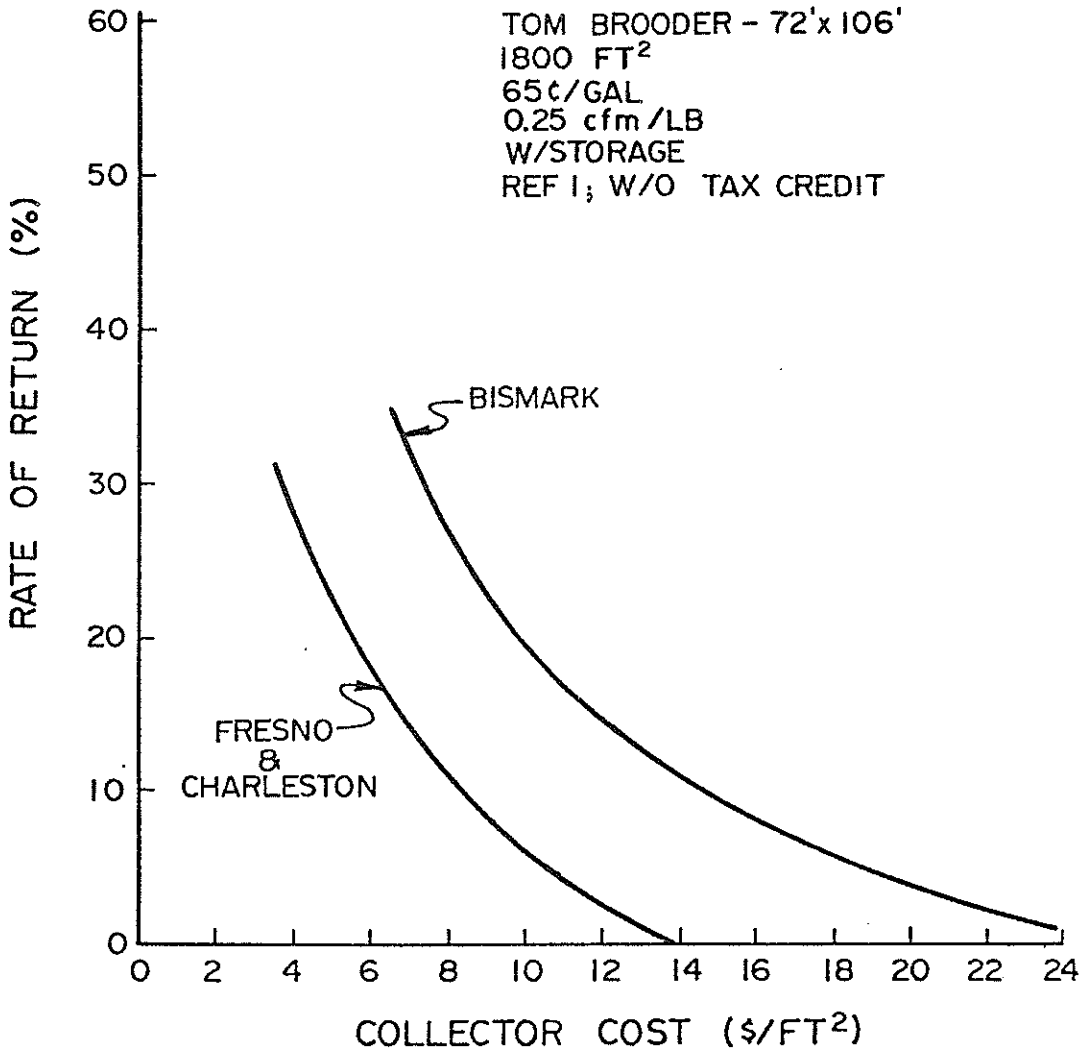

Figure 9. A geographical relationship in rate of return gives relative attractiveness of applications.

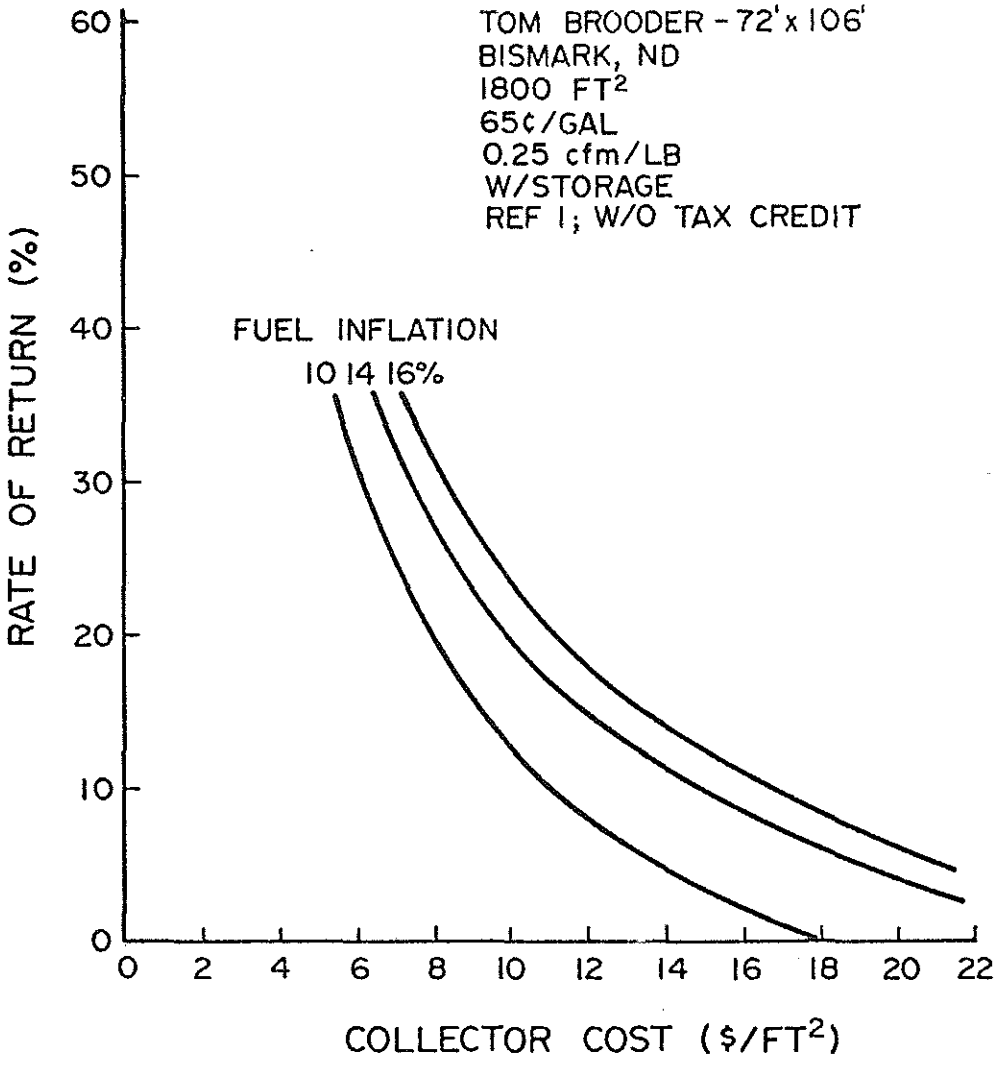

Figure 10. A slight variation of one of thirteen assumed parameters significantly affects the rate of return.

MANAGING TOMS FOR MAXIMUM SEMEN PRODUCTION

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INTRODUCTION

Managing the male turkey for maximum high quality semen production includes everything done to and for that turkey from hatching until the end of the breeding season. The hatching egg industry lives and dies by fertility and hatchability of the eggs produced. Thus nothing should be too good for the toms that produce the semen necessary for high fertility. Because of increased demand for heavier turkeys with increasing processing yields, breedermen are forced to maintain turkey breeder toms of increasing size which leads to a number of problems such as:

(1) Toms may begin producing semen as early as 22 weeks of age. Since the early maturing toms are usually poor semen producers late in the breeding season, they need to be held back either by light control programs, feed restriction, or a combination of both.

(2) Heavy breeder toms are difficult to handle and collect semen from.

(3) Leg weakness problems are more pronounced resulting in high morbidity and mortality.

(4) A large amount of feed is required to maintain full-fed breeder toms.

The ideal tom breeder management program should include the following:

(1) Feeding commercial growing rations to the age at which their full genetic growth and conformation are expressed. Selecting males at an early age results in some loss in this genetic potential via selection mistakes.

(2) Lighting and feeding programs should be initiated early enough to minimize leg problems and to reduce the final weight of the breeder male. These management procedures should result in high quality, high volume ejaculates at 31-32 weeks of age when egg production begins. Of course the feeding and management programs should be cost effective.

You will note there may be some conflict between feeding to allow maximum expression of genetic growth potential in (1) above and restricting growth through feeding programs early enough to minimize leg problems as noted in (2). We now turn to programs that have been suggested and/or are now being used.

Program 1

Full-feed toms and use light to control the time semen production begins

A study conducted at the Ohio station in 1976 confirmed the lighting procedure then being used by many turkey breeders. In this study Nicholas male line turkeys were hatched January 9, 1976. They were brooded under infra-red heat lamps to four weeks of age and then were maintained under 1.5 foot candles (16.1 lux) and 12-hour light days until May 28 when they were 20 weeks of age. At that time they were housed in individual tom cages. The cages had solid floors with wood shavings litter. Four cages were placed in each of 8 rooms with separate lighting and ventilation. There were two rooms on each light treatment. The light treatments from age 20 weeks were as follows:

1. 12 hours of light per day at an intensity of 5.4 lux (0.5 foot candle) from 20 weeks through 60 weeks of age.

2. 12 hours of light at 5.4 lux from 20-26 weeks of age and then 14 hours of light at 5.4 lux from 26-60 weeks of age.

3. 12 hours of light at 5.4 lux from 20-26 weeks of age followed by 14-hour days and 10.8 lux (1 foot candle) from 26-60 weeks of age.

4. 6 hours of light and 5.4 lux with changes to 12 hours and 5.4 lux at 26 weeks, 12 hours and 10.8 lux at 32 weeks, 13 hours and 10.8 lux at 38 weeks, 13 hours and 16 lux (1.5 foot candles) at 44 weeks, and 14 hours and 16 lux at 50 weeks. The 14 hours of light per day and 16 lux intensity were continued on to 60 weeks of age when the study was terminated.

Individual semen samples were collected at 4-week intervals beginning at 32 weeks of age. At the time semen was collected a molt rating was made. The rating was 1-5 with 1 being no molt and 5 being nearly complete molt on both the rear and the back of the bird. Volume was determined by drawing the sample into either a 1/4 or 1/2 ml syringe. Density (a measure of sperm numbers) was determined by a 1:1000 fold dilution and optical density read on a Gilford 300N microspectrophotometer at 540 mμ according to the method of Brown et al. (1970).

When the males were 31 weeks of age a fertility trial was run in which four hens were inseminated with semen from each male producing semen. Each hen was inseminated with 0.025 ml of neat semen on Monday and Wednesday of the first week and at two week intervals for a 9-week fertility trial. The hens were Ohio egg line, 16-week line, and low adrenal line turkeys. Toms on each light treatment were randomly assigned to equal numbers of females from each line. The hens used had been in egg production for 14 weeks at the beginning of the trial. The fertility trial began on August 9; thus it was conducted at a time in the summer and the reproductive cycle of the hens when fertility and hatchability are usually mildly depressed.

Semen quality and molt ratings are given in Tables 1 and 2. Light treatments 1 (12 hours light at 5.4 lux) and 2 (12 hours light at 5.4 lux from 20-26 weeks followed by 14 hours of light at 5.4 lux from 26-60 weeks) produced significantly higher semen volume than treatments 3 and 4 where both day length and light intensity were increased. Semen volume and density did not differ between treatments 1 and 2.

Light treatments had no influence on molt ratings. It should be noted that at 20 weeks of age these males were placed on a light schedule where the intensity was reduced from 16 lux to 5.4 lux. At 26 weeks almost all birds were in a rather complete molt. Subsequent studies indicate toms can be grown on a 12-hour light day and 5.4 lux thus avoiding this molt and come into semen right on schedule at 30 to 32 weeks of age.

The results of the fertility trial are shown in Table 3. Male light treatments had no effect on fertility. Although the fertility appears a bit low it is normal for this age of hen during the summer and fall months when two week insemination intervals are used. It should also be noted that during this time of the year we are routinely setting only a few hundred eggs per week in a 10,000 capacity Robbins incubator. Under these conditions hatchability is lower than when the incubator is completely filled with eggs from only one age and strain of turkeys. However, all treatments were subjected to the same conditions; thus the results are a valid reflection of the effect of light treatment on fertility and hatchability.

Advantages of this system are (1) toms can be maintained under light intensity of 5.4 lux and 12-hour light days before sexual maturity is attained and throughout the breeding season resulting in quiet birds, low mortality and morbidity due to reduced fighting. (2) High volume, high quality semen is also produced with this procedure.

Disadvantages are that this procedure requires a well-designed, environmentally-controlled house. For best results fan capacity should be 2 CFM per pound of turkey and 6-10 square foot per male housed. Trying to institute this procedure in an over-crowded, poorly ventilated house usually leads to disaster.

Program 2

Controlled feeding of turkey breeder toms (Krueger, Turkey World, May/June, 1979; Krueger, Ph.D. Thesis, 1976; Krueger et al., 1977)

Krueger (1977) presented data indicating restricting daily feed consumption to approximately 50% of the full-fed males from 18 weeks of age resulted in a 30% reduction in body weight at 30 weeks of age; body weight for restricted-fed males was significantly less than that of full-fed males through 65 weeks of age. Regression analysis of semen volume indicated that restricted-fed males receiving 15 hours of light, and full-fed males receiving either 12 or 8 hours of light per day, produced increasing semen volume over a 30-week collection period (from 32-62 weeks of age), while full-fed males receiving 15 hours of light per day exhibited a decline in semen production. Fertility of eggs from BBW turkey hens, inseminated with semen from restricted-fed males was significantly ($P<.05$) higher than fertility following inseminations from full-fed males receiving 15, 12, or 8 hours of light per day.

The seasonal decline in semen quality and quantity, often experienced in turkey breeder males, was prevented through the use of the feed restriction program. Males in the restricted-fed group were easier to handle and ejaculate than the full-fed groups. Restricting feed intake resulted in a 55% saving in feed consumed.

Based on these results Krueger (Turkey World, 1979) conducted field trials and developed a recommended procedure for controlled feeding of turkey breeder toms. This program consists of selecting breeder toms at 16-18 weeks of age prior to vocalization and strutting and immediately moving the selected males to breeder pens where feed intake is controlled. Birds should be allowed a minimum of 12 linear inches per bird. They are fed a pelleted simple corn-soybean ration containing 1450 Kcal M.E./lb, 0.80% calcium, and 0.70% total phosphorus with breeder vitamin fortification. The calculated protein content was approximately 16%. Beginning at 16 to 18 weeks of age and continuing for two weeks, control-fed toms are fed once daily 0.55 pounds per bird. Normally the control-fed toms will lose weight during the first two weeks. Thereafter, if necessary, adjust the consumption weekly to allow approximately a 0.25 to 0.50 pound increase in average body weight per week.

Because controlled feeding of turkey breeder toms tends to delay sexual maturity, lighting is not as critical as for full-fed toms. He generally suggests 14 hours of light per day at one foot candle (10.6 lux).

The advantages of this program are that an environmentally controlled house is not necessary, the weight of the toms is controlled thus resulting in fewer leg problems, less morbidity and mortality, and a high volume, high quality semen throughout the breeding season. In fact, males subjected to this program often will produce good quality semen for two successive breeder hen flocks. There is a significant reduction in feed costs. Disadvantages are the necessity to monitor weights and to hand feed known quantities of feed each day.

Program 3

Self-imposed reduction in feed restriction by feeding 17% protein diet from 8 to 28 weeks.

After 28 weeks of age dietary protein is reduced to 8 percent without adverse effects on semen quality or quantity (Helene Cecil, Turkey World, 1981; Poultry Science, 1981). Dr. Cecil found that when males were fed dietary protein levels (11, 13, 15, and 17%) from 8 to 52 weeks of age, that only those fed 17% protein diets were producing semen at 30 weeks of age. Males fed 11, 13 or 15% protein diets did not reach 85% semen production until 43, 39, and 37 weeks of age. She calculated that breeder turkey males need to gain 1.2 pounds per week from 12 weeks on to reach sexual maturity at 30 weeks of age.

Advantages are that feeding the 17% and 8% protein diets resulted in self-imposed restriction on feed consumption that caused a slower rate of body weight gain. In addition, the birds had fewer leg problems and semen collection was easier.

Compared with the restricted feeding program, feeding a low protein diet has the advantage of eliminating weighing the turkeys weekly, calculating the feed to be fed, and weighing the feed daily. In addition, feed costs were reduced because of decreased feed consumption and the lower cost of the diet formulation.

A major disadvantage is that the males were placed on the 17% protein diet at 8 weeks of age well before their genetic potential for growth and conformation could be expressed at market age. This would have the effect of reducing the selection pressure for large growth males with the best conformation and strong legs.

A Theoretical 4th Program

Self imposed feed restriction by feeding a low protein diet at or near market age after the males full genetic potential for growth and conformation has been fully expressed.

This would eliminate slower growing males and males that develop poor legs as market age approaches. This procedure should result in higher quality poults because of the greater genetic potential of the breeder toms.

In 1981 we set out to both dip and inject eggs from all our genetic stock in an attempt to eliminate Mycoplasma meleagridis. We succeeded; however, the treatment greatly reduced hatchability. As a result, Dr. Nestor was obliged to save poults from 12 weekly hatches to obtain enough poults to carry on his usual genetic selections. He normally saves 4 weekly hatches. Thus, this year we have a unique opportunity to assess the interactions of age and low protein diets on semen production, leg weakness, mortality, morbidity, etc.

Ohio 16-week leg line (16-L) and Ohio 16-week egg line (16-E) are being used for this study. From 8 to 16 weeks of age the turkeys were grown on pasture ranges. At 16 weeks of age each hatch was housed in a building with small windows on one side and were given natural light only. When the last hatch was 16 weeks old and was housed on November 9, all ages ranging from 16 to 27 weeks were intermingled in 10 pens. A total of 180 (both lines combined) toms were placed on experiment. Eight square feet of floor space was provided per tom. The birds were all weighed and their leg condition rated. This rating was done by the farm foreman when the toms were released in the pen after being weighed. Each male was given a rating from 1 to 5 with 1 being very good legs and 5 being very bad legs so that the male can hardly walk. Ratings 2, 3 and 4 represent intermediate severity of leg weakness. Half (5 of 10 pens) were placed on 11% protein (1405 cal/lb) feed and the other 5 pens were given 14% protein (1405 cal/lb) feed. It should be noted that all control toms 20 weeks or older received the standard Ohio grower rations to 20 weeks of age. However, the 16, 17, 18, and 19 week old turkeys (Treatment 2) at the beginning of the study were changed to the 14% tom breeder ration at the age when the older birds were still on 16.5% protein growing rations. At 4-week intervals all birds will be weighed, legs rated, and semen volume and density measured. The males in the two lines selected for breeders will be used to inseminate selected breeder hens for 12 weeks; thus a measure of the fertilizing capacity of the semen for the two treatments will be made.

All males on both treatments were placed on stimulatory light (5.4 lux and 12 hours per 24 hour day) as the study began.

At the time of writing this paper, the data after 4 weeks on 11% and 14% protein rations were available (Table 4). Although these data are very

preliminary, it is apparent that 11% protein is resulting in decreased weight gains. In all but the oldest group (25-27 weeks at the beginning of the experiment) there was no increase in leg problems for males on 11% protein. By contrast, the older group on 14% protein rations experienced an increase in leg problems. It appears that large white males can be selected as late as 20 to 24 weeks, placed on a low protein diet at that time, and still reduce subsequent leg problems. Previous work indicates that full-fed males need stimulatory light for 8 weeks to reach maximum semen volume. You will note in Table 4 that after 4 weeks of stimulatory light only the older birds produced semen and that the males fed 11% protein are coming into semen production slower than birds fed 14% protein. Thus, when low protein rations are fed stimulatory light (5.4 lux and 12 hours) should probably begin no later than 20 weeks of age to insure full semen production by 30 weeks of age.

SUMMARY

Principles of breeder tom management have been reviewed. No cookbook procedures are or should be suggested to cover all strains of turkeys and environmental conditions. Each hatching egg producer needs to develop a management system that works best for his or her situation. The following guidelines for the development of a management system are suggested.

1. Grow the toms on commercial turkey growing rations to or near market age to allow for full genetic potential for growth, conformation, and strong legs to be expressed.

2. At or near market age toms should be selected. Severe selection pressure should be applied for size and conformation. All birds with poor legs should be rejected.

3. At the time of selection the toms should be placed in breeder pens, preferably in small groups, given stimulatory light (5 lux and 12 hours is sufficient) and placed either on a controlled feeding program or a low protein ration that results in reduced weight gains to 30 weeks of age. The 11% and 1400 Kcal M.E./lb currently being tested at the Ohio station has been used successfully in commercial flocks.

4. Based on the work of Dr. Cecil, a further reduction to 8% protein at 30 weeks of age will maintain semen production throughout the breeding season.

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Table 1. Effect of light treatments on semen volume, density and molting.

Age in Weeks		Light Treatments 1 vol	1 conc	1 molt	2 vol	2 conc	2 molt	3 vol	3 conc	3 molt	4 vol	4 conc	4 molt
(1)	32	0.402	0.116	4.33	0.408	0.186	3.40	0.372	0.137	3.50	0.252	0.135	2.83
(2)	36	0.465	0.131	3.33	0.470	0.122	3.00	0.432	0.128	2.67	0.327	0.150	4.00
(3)	40	0.417	0.130	3.67	0.366	0.142	4.00	0.367	0.138	3.33	0.333	0.131	2.83
(4)	44	0.283	0.134	4.00	0.264	0.127	4.40	0.242	0.123	4.17	0.258	0.134	3.00
(5)	48	0.357	0.133	3.50	0.328	0.152	4.20	0.255	0.120	3.83	0.285	0.158	2.67
(6)	52	0.327	0.116	3.00	0.346	0.156	3.20	2.242	0.108	3.33	0.235	0.108	3.17
(7)	56	0.433	0.124	3.17	0.356	0.126	3.40	0.272	0.111	3.00	0.273	0.117	3.50
(8)	60	0.382	0.104	3.00	0.363	0.121	3.00	0.235	0.089	3.00	0.297	0.113	3.67
$\bar{x}$		0.383	0.124	3.50	0.363	0.142	3.58	0.294	0.119	3.35	0.282	0.131	3.10

Table 2. Tukey's test for significance applied to effect of light treatments on semen characteristics and molt rating.

Trait Measured	Light Treatments 1	2	3	4
Volume	0.383a	0.363a	0.294b	0.282b
Concentration (O.D.)	0.124ab	0.142a	0.119b	0.131ab
Molt Rating	3.500a	3.575a	3.354a	3.104a

Means followed by different letters are significantly different ($P<.05$).

Table 3. Effect of male light treatment on fertility and hatchability over a nine week period.

Male Light Treatment	% Fertility	% Hatch of Fertile Eggs	% Hatch of Total
1	82.6	70.3	58.1
2	83.0	73.0	60.6
3	80.2	75.0	60.3
4	79.5	75.2	59.9

Table 4. Effect of 11% protein ration on large white turkeys starting at ages ranging from 16 to 27 weeks.

Age in Weeks	Treatment 1			Treatment 2		
	Weight	Leg R	Semen	Weight	Leg R	Semen
16, 17, 18*	23.3	1.79	0/15	23.9	1.79	0/17
20, 21, 22	26.8	1.67	0/15	28.9	1.86	0/17
19, 20, 21*	27.2	2.00	0/14	28.0	1.92	0/13
23, 24, 25	31.9	1.99	0/14	33.4	2.52	0/13
22, 23, 24*	32.1	1.73	0/26	33.0	2.00	0/27
26, 27, 28	35.6	1.66	3/26	38.3	2.47	7/27
25, 26, 27*	33.9	1.84	0/32	33.8	2.16	0/33
29, 30, 31	36.5	2.33	2/32	39.2	2.40	7/33

*Age when 11% protein diets were started.

INFLUENCE OF HE VIRUS ON AVIAN IMMUNE SYSTEM

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INTRODUCTION

Hemorrhagic Enteritis (HE) is an acute enteric viral disease of four week or older turkeys. It is caused by an Adenovirus. HE is fairly universal wherever turkeys are raised and is at least in ten states of the U.S. It is also recognized in other countries that raise turkeys. Signs are depression, bloody droppings and death which occurs rather rapidly after onset of the disease.

Some viruses have long been known to cause impairment of host immunologic competency. Immunosuppression caused by some oncogenic (tumor producing) and non-oncogenic viruses has been reported[3]. It is now widely recognized that many viruses can directly affect cellular immunity. Viruses reported to affect cellular immunity include Influenza Virus, Mengovirus and Newcastle disease Virus[4]. Cellular (mitogenic) and humoral (plaque forming) responses were examined in turkeys after experimental infection with Hemorrhagic Enteritis Virus (HEV). The results of this experiment and the influence of HE virus on the immune system of turkeys is discussed here.

MATERIALS AND METHODS

Turkey poults obtained from a specific pathogen free flock were raised in strict isolation and infected with purified HE Virus at four weeks of age.

The cellular immune response was examined by lymphocyte stimulation assay. The mitogens used for lymphocyte stimulation were concanavalin-A (conA) and phytohemagglutinin (PHA). At weekly intervals heparinized blood samples were collected beginning one week post-infection. The whole blood assay (2) was used. Blood samples from turkeys exposed to HEV and unexposed controls were stimulated with mitogens for 48 hours at 41°C. At the end of 48 hours, the radioisotope 125_{IUDR} was added and incubation continued further for 6 hours. At the end of this period cells were harvested and its radioactivity was counted in Gamma Counter.

A hemolytic plaque assay originally described by Jerne and Nordin[1] was modified and used for the detection of antibody producing cells in HE infected turkeys. At one week post-infection both infected and uninfected turkeys were given sheep red blood cells (SRBC) intravenously. The antibody response to SRBC in splenic cell suspensions of both groups was determined.

RESULTS

The results indicated that there was a significant difference in the mitogenic response between HE infected birds and noninfected controls (Figure 1 and 2). Mitogenic response for ConA and PHA increased with time in the uninfected controls. Whereas in the infected birds there was a drop in the response up to 5 weeks post-infection after which the response started increasing gradually.

The hemolytic plaque assay showed a significant difference in the number of plaques between HE infected birds and noninfected controls. Noninfected controls had higher number of plaques consistantly. There was a decreased ability of HE infected turkeys to produce SRBC antibodies. The greatest inhibition was seen in turkeys 19 days after exposure to HEV (Figure 3).

DISCUSSION

Viral invasion of reticuloendothelial system takes place in HE infections. There might be an effect on proliferative potential of lymphocytes by HE virus. This could very well account for the lower mitogenic response seen in the infected birds.

It was also evident from the findings of the present study that the ability of the infected birds to respond to SRBC was reduced. There were fewer plaque forming cells (PFC) in the infected groups. The PFC response is considered to be an indicator of humoral antibody production. Many explanations have been given to explain how viruses which infect cells of the immune system such as HEV could depress the antibody production. They may do so by altering the uptake and processing of antigens.

The important thing derived from the results of this study was HEV induces a significant immunosuppression. The depression of T cell activity was more pronounced than that of B cells.

The consequences of immunosuppression can cause progression of currently active infections or predispose the host to other infections such as Colibacillosis, Avian Influenza Virus or Newcastle Disease Virus. This may also significantly alter the birds response to vaccines such as Fowl Cholera or Newcastle Disease Vaccine.

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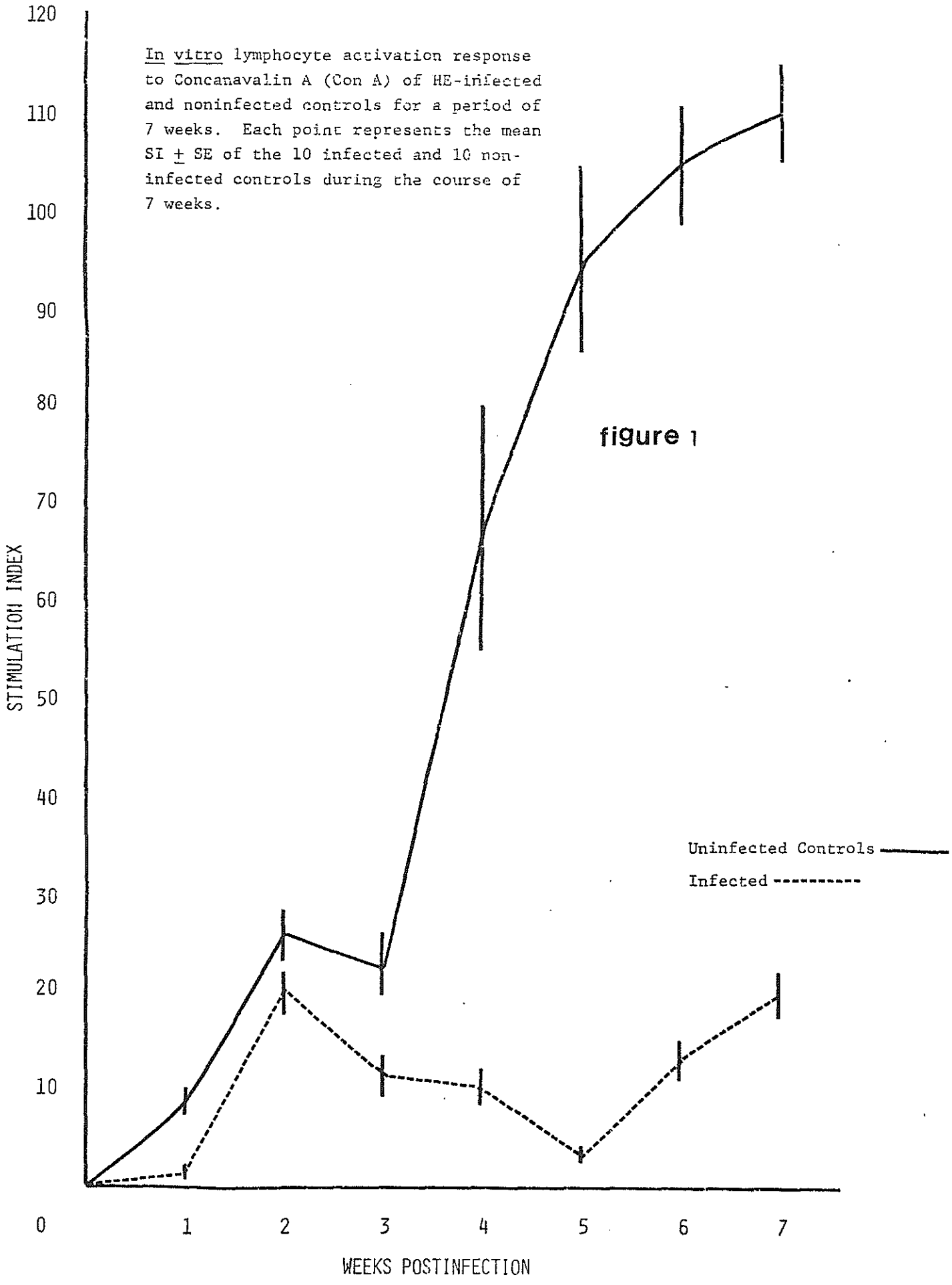


In vitro lymphocyte activation response to Concanavalin A (Con A) of HE-infected and noninfected controls for a period of 7 weeks. Each point represents the mean SI ± SE of the 10 infected and 10 non-infected controls during the course of 7 weeks.

figure 1

In vitro lymphocyte activation response to Phytohemagglutinin (PHA) of HE-infected and noninfected controls for a period of 7 weeks. Each point represents the mean SI ± SE of the 10 infected and 10 noninfected controls during the course of 7 weeks.

figure 2

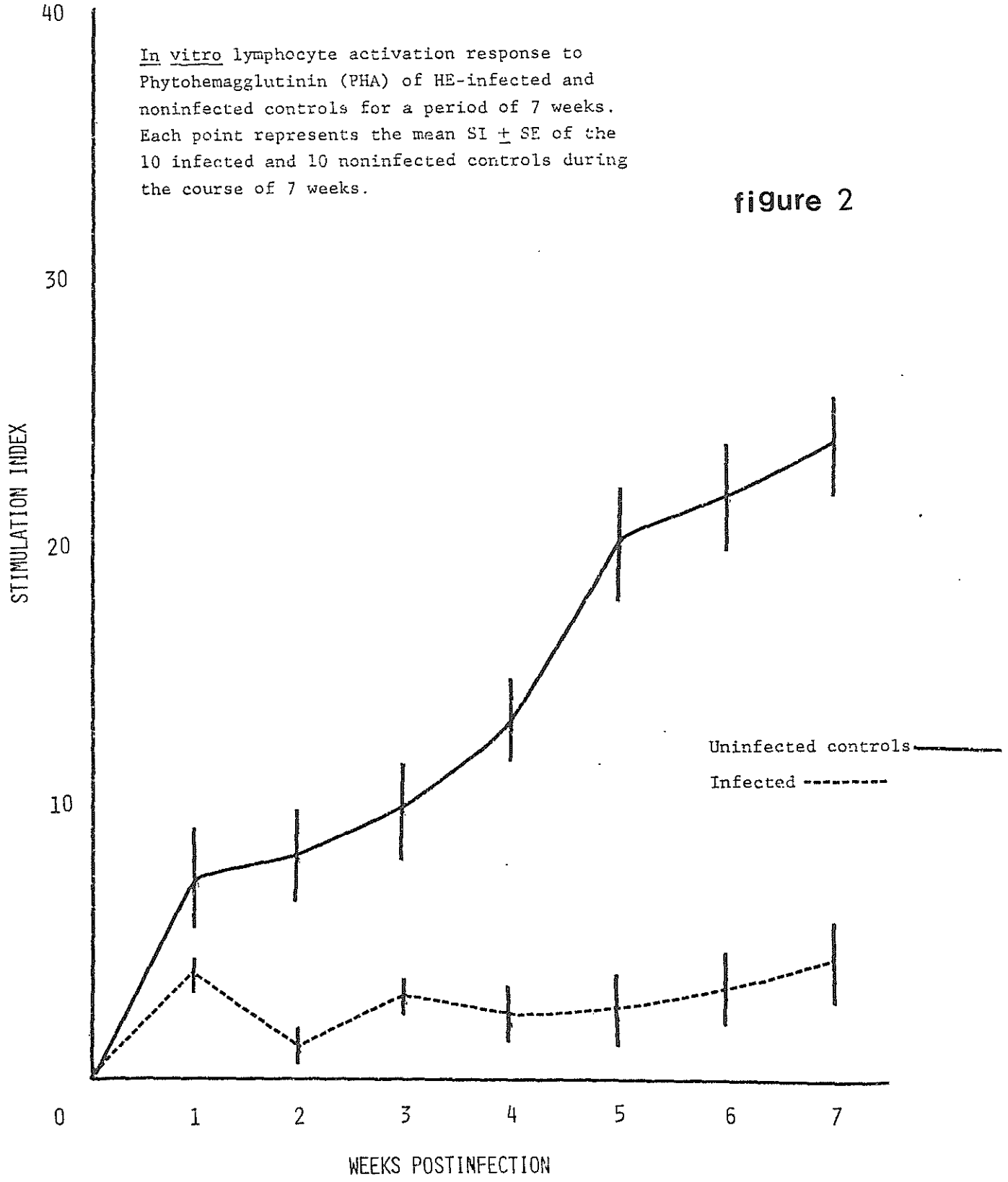
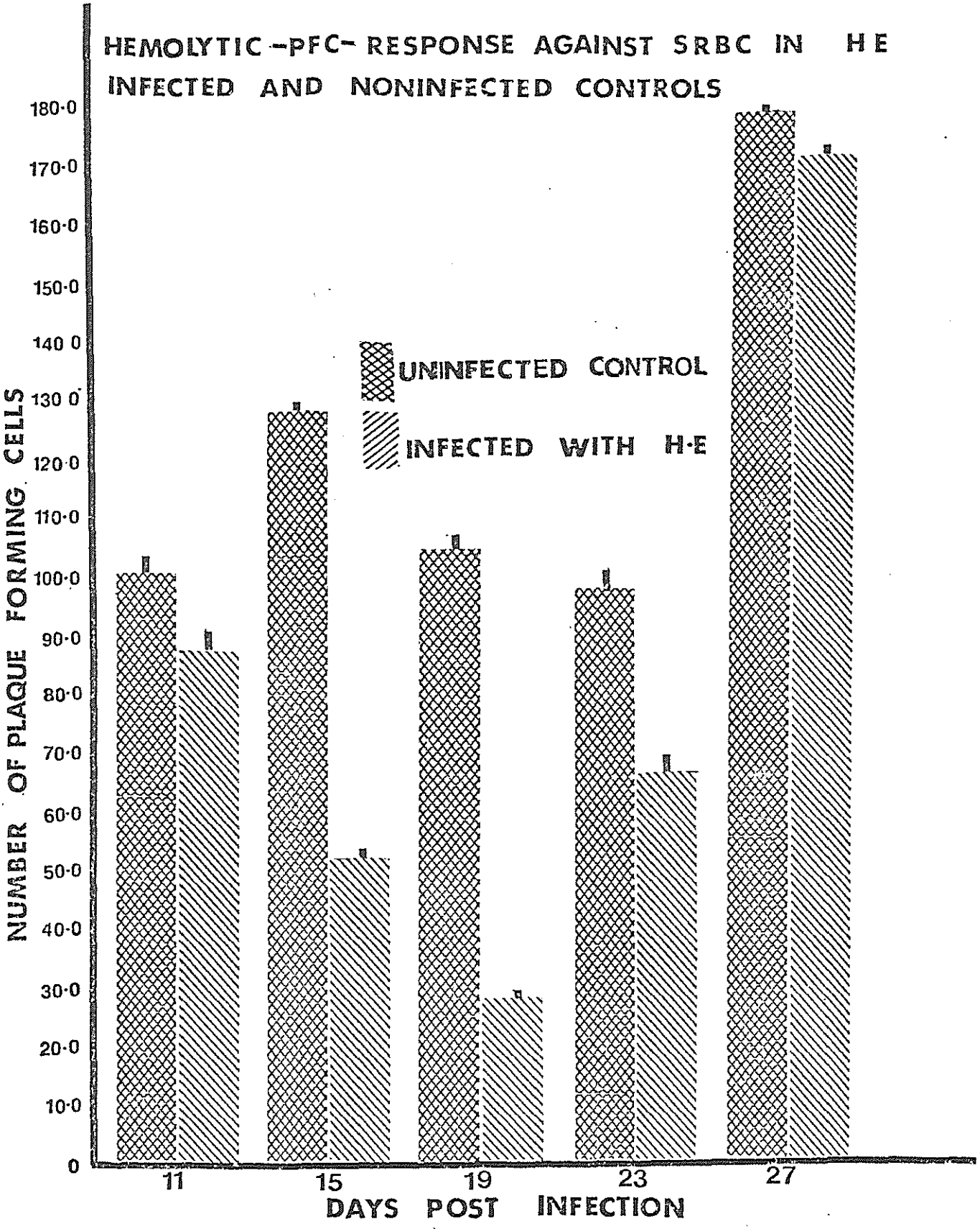


FIGURE 3


HEMOLYTIC -PFC- RESPONSE AGAINST SRBC IN HE INFECTED AND NONINFECTED CONTROLS
NUMBER OF PLAQUE FORMING CELLS
180·0
170·0
160·0
150·0
140 0
130 0
120·0
110·0
100·0
90·0
80·0
70·0
60·0
50·0
40·0
30·0
20·0
10·0
0
UNINFECTED CONTROL
INFECTED WITH H·E
11
15
19
23
27
DAYS POST INFECTION

EFFECT OF AMMONIA ON AVIAN RESPIRATORY SYSTEM

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INTRODUCTION

Environmental factors such as dust, temperature, humidity and irritant gases have been shown to influence the survival or infectivity of some microbial agents or the pathogenesis of some diseases. Managemental practices such as confinement rearing of turkeys and reuse of litter have led to conditions favoring the continual release of ammonia from the litter into the atmosphere of the birds' environment. Ammonia is a colorless, highly irritant gas. Reduced ventilation can result in the accumulation of this gas in large amounts. One of the research interests of the Avian Disease Research group at the College of Veterinary Medicine was to study the effects of exposure to ammonia on the influence of respiratory E. coli infection in turkeys. This work was done in cooperation with the Department of Agricultural Engineering.

MATERIALS AND METHODS

Two thousand eighty small white male Wrolstad turkeys were used in this experiment. They were distributed in two separate buildings. Building 1 had eight pens and building 2 had four pens. The pen density was 1.4 ft^2 per bird. The experimental variables were air flow and ammonia concentration as shown in figure 1.

At two weeks of age the turkeys were aerosolized with a live avirulent E. coli vaccine (serotype 078). Some groups were challenged by aerosolization two weeks post vaccination. The birds were challenged with virulent strain of E. coli (serotype 078). Controls were maintained for each of these treatments.

Five birds in each group were sacrificed ½ hour, 1 and 2 weeks post vaccination and at 1, 2, 3, and 4 and 5 days post challenge. Tissue samples were pooled from lungs, air sacs and liver in each group and plated on selective media for quantitation of E. coli 078.

Tracheal and lung specimens were collected each time when birds were sacrificed. The tissues were examined for histopathological changes and for ultra structural changes under Scanning Electron Microscope.

At weekly intervals blood was collected from each group to determine their immunocompetency. A lymphoblastogenesis assay was done using the whole blood and the radioisotope 125_{IUDR}.

RESULTS

In turkeys exposed to ammonia more E. coli was detected in lungs, air sacs and liver. Turkeys unexposed to ammonia had better clearance of E. coli from their system: Vaccination seemed to have a beneficial effect on improving the rate of clearance of E. coli in birds not exposed to ammonia. A highly significant number of turkeys exposed to 40 ppm of ammonia had air sac lesions.

Histopathology revealed mild, acute inflammatory changes in tracheas of birds exposed to 40 ppm of ammonia. The tracheal epithelium was hyperplastic and had a tendency to form goblet cells with mucus secretion. The tissues of unexposed birds appeared normal.

Scanning electron microscopy of the luminal surface of the tracheas revealed areas of ciliary distruction and proliferation of goblet cells in birds exposed to 40 ppm of ammonia. The severity of this damage appeared to be related to the time of exposure to ammonia. The deciliation of the epithelium was seen by 5 weeks of exposure. The functional integrity of cilia was also affected.

Large areas with mucus accumulation were observed. The tracheal tissues of birds unexposed to ammonia appeared normal.

There was a decreased lymphoblastogenesis to non-specific stimulation by mitogens in the birds exposed to ammonia compared to the unexposed birds.

DISCUSSION

Different aspects of airway clearance have to be taken into consideration in order to recognize that a break in the defense mechanism may take place at one or many levels. It was evident from this experiment that turkeys exposed to 40 ppm of ammonia suffered from mild, acute inflammatory changes in their tracheal epithelium. An accumulation of mucus was observed. This was probably due to irritation of the epithelium by the ammonia resulting in increased goblet cell activity. Scanning electron microscopy revealed damage to the structural integrity of tracheal epithelium. This led to a reduction in the efficiency of the mucociliary function facilating the infection by respiratory pathogens.

Birds exposed to ammonia had lower blastogenesis which reflects lowered immunocompetency in these birds. Immunosuppression can further enhance the pathogenesis of a respiratory pathogen.

Improved ventilation is necessary to avoid the natural buildup of the ammonia in the barns.

FIGURE - 1

AIR FLOW AND AMMONIA CONCENTRATIONS

1. BLDG. A

WEST 4 PENS VENTILATED AT FOUR AIR CHANGES/HOUR

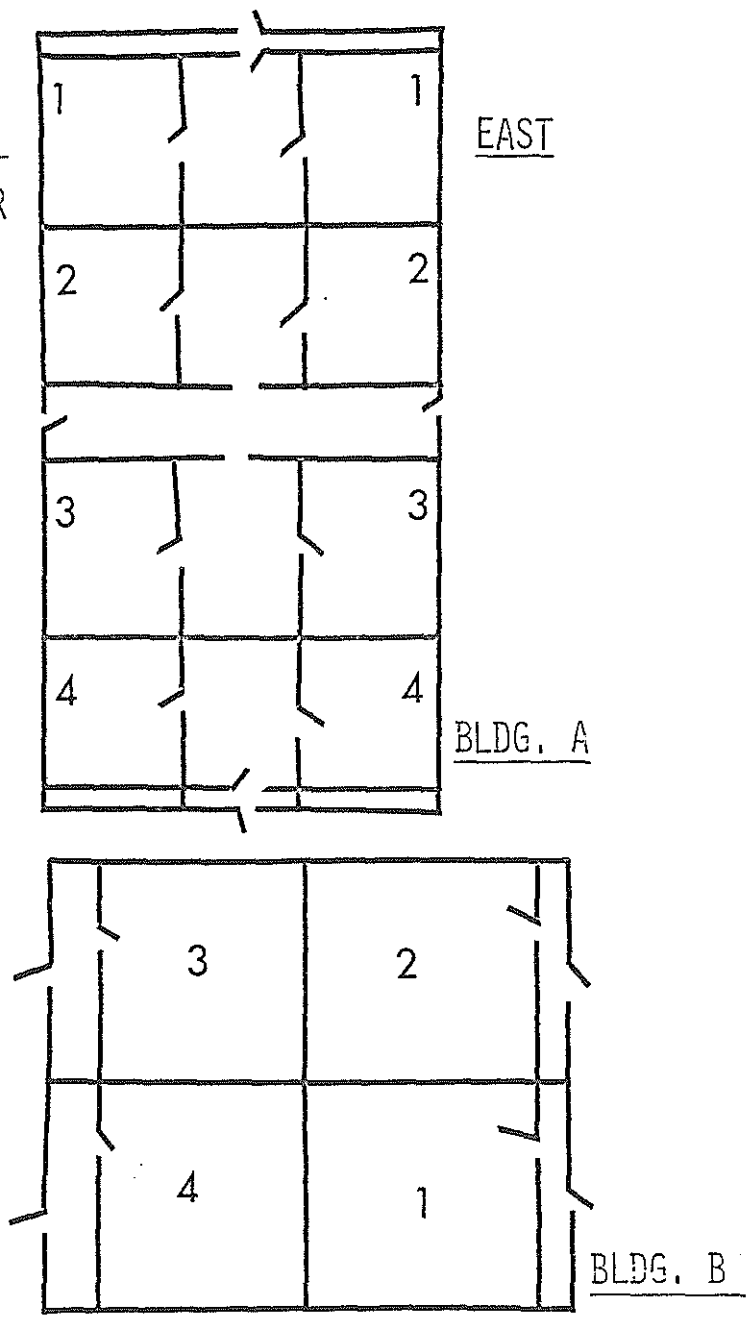
EAST 4 PENS VENTILATED AT ONE AIR CHANGE/HOUR

ALL PENS IN "A" HAD FLAT PLATE ORIFICES FOR AIR FLOW CONTROL

IN BUILDING "A" AMMONIA WAS INTRODUCED AT 45 ML./MINUTE

2. BLDG. B

VENTILATION ADJUSTED AS REQUIRED FOR GOOD AIR QUALITY



AVIAN INFLUENZA IN MINNESOTA - AN UPDATE

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INTRODUCTION

Avian Influenza (AI) is a viral disease affecting primarily the respiratory system of many kinds of poultry and birds. All influenza viruses encountered so far in the infection of commercial poultry are Influenza type A viruses belonging to Orthomyxovirus group. All Avian Influenza viruses (AIV) have the ability to hemagglutinate chicken red blood cells. The virus is excreted in high concentrations in the nasal secretions and feces of infected birds. The virus is very sensitive to heat and chemicals such as formalin (0.05 to 0.1%). It remains infective at least 30 days in feces at 4°C. The virus has been isolated from water troughs on turkey ranges and pond water where a high concentration ofducks were observed. AI viruses fail to produce any clinical signs or detectable immune response in waterfowl. The nature of infection in waterfowl seems to be primarily intestinal[1,2,4]. The several influenza A virus strains in nature are classified based on their Hemagglutinin (H) and Neuraminidase (N) surface antigens, according to the World Health Organization's recommendation. Currently 13 H antigens and nine N antigens have been identified[5].

The severity of the disease in turkeys depends on the age of the birds when infected, the virulence of the strain involved and the presence of other secondary viral and/or bacterial invaders[3]. AIV infected breeder flocks experience a drastic drop in egg production. AI has been a persistent problem in Minnesota turkeys since 1966, when it was first identified. It is becoming an increasingly important problem facing the turkey industry. The 1978-79 AI outbreak became the most extensive and costly outbreak in the history of the disease in Minnesota. Approximately 130 market flocks involving 2,317,989 turkeys and 11 breeder flocks involving 27,680 hens were affected[6]. During the past four years (1978-81) the turkey industry in Minnesota alone lost over 8 million dollars due to AI.

The University of Minnesota, Avian Disease Program is researching several aspects of the problem.

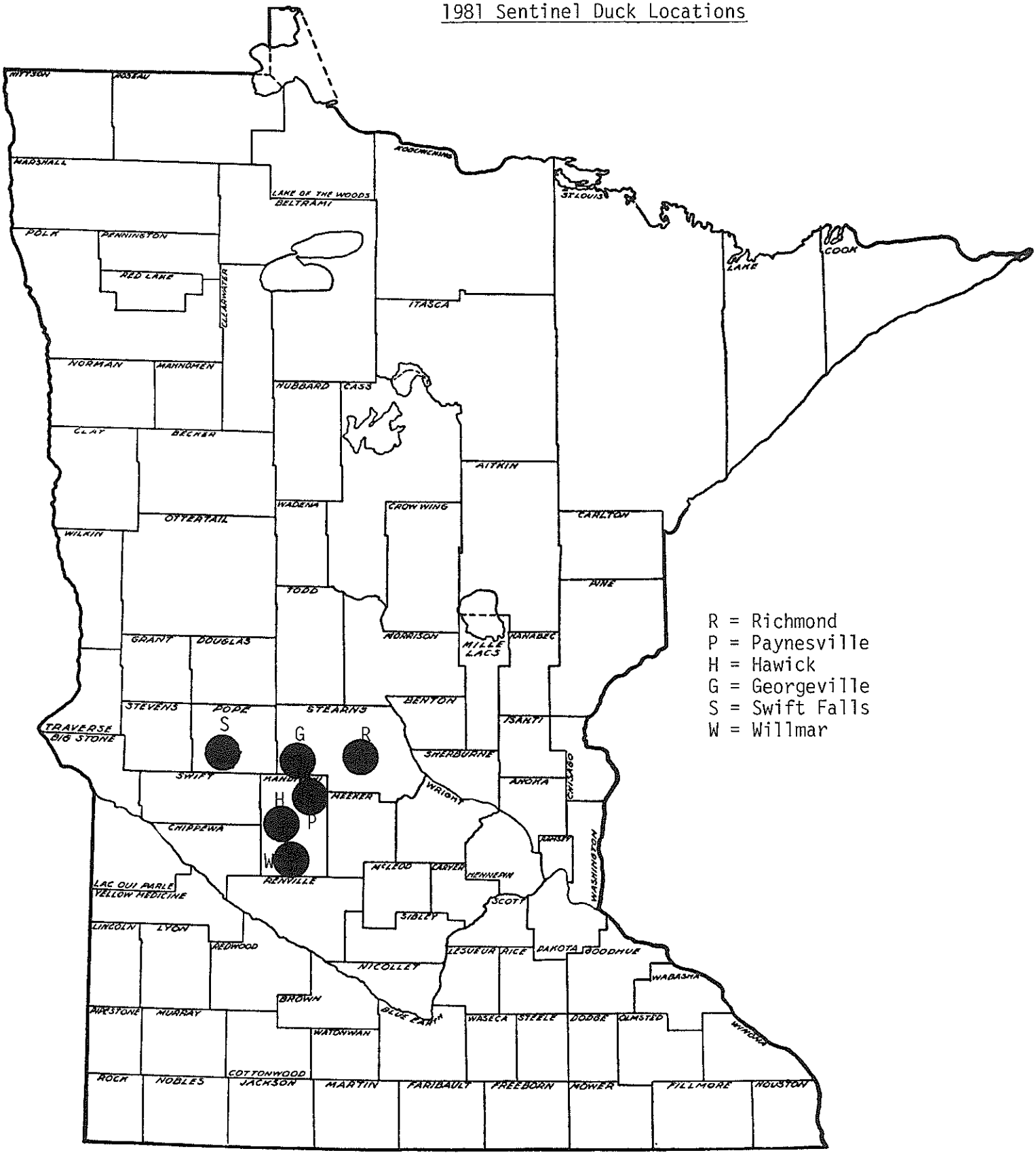
EPIDEMIOLOGY

a. Sentinel and Messenger Duck Study Model

An epideminological study was carried out in six locations in Minnesota (See Figure 1), in 1981. The objective of this study, which began in April, 1980, is to identify the factors involved in the transmission AIV from wild waterfowl to domestic poultry.

FIGURE 1

1981 Sentinel Duck Locations



Day-old, pinioned,mallard ducklings were grown in confinement until 6-weeks-of-age. Then 25 ducks were caged at each of the six locations (sentinels). Some were later released (messengers). Cloacal swabs were collected weekly beginning the middle of May until the end of October. Cloacal swabs and serum samples were collected simultaneously from turkeys on range from nearby farms. A variety of hemagglutinating (HA) agents were isolated. The results are shown in Table 1.

A total of 1611cloacal swabs were collected and 137 HA virus isolations were made (8.5% isolation rate). The isolation rate for each location is given in Table 2. The isolation rate in 1981 was lower when compared to last year.

TABLE 1

HEMAGGLUTINATING AGENTS ISOLATED AND IDENTIFIED FROM SENTINEL DUCKS AND TURKEYS

A/Mallard Duck/MN/1311/81	H8N4 (Hav8Nav4)
A/Mallard Duck/MN/1313/81	H10N7 (Hav2Neg1)
A/Mallard Duck/MN/1325/81	H12N4 (Hav10Nav4)
A/Mallard Duck/MN/1359/81	H3N8 (Hav7Neg2)
A/Mallard Duck/MN/1370/81	H1N1 (Hsw1 N1)
A/Mallard Duck/MN/1383/81	H6N1 (Hav6 N1)
A/Mallard Duck/MN/1390/81	H6N6 (Hav6Nav1)
A/Mallard Duck/MN/1450/81	H4N2 (Hav4N2)
A/Mallard Duck/MN/1473/81	H5N2 (Hav4N2)
A/Mallard Duck/MN/1490/81	H1N2 (H1N2)
A/Mallard Duck/MN/1498/81	H11N9 (Hav3Nav6)
A/Mallard Duck/MN/1318/81	H7 N3 (Hav1Nav2)
A/Mallard Duck/MN/1465/81	H3 N2 (Hav7 N2)
A/Seagull/MN/1352/81	H13N6 (Hav11Nav1)
A/Pigeon/MN/1407/81	H1 N1 (Hsw1N1)
Mallard Ducks and Turkeys	New Castle Disease Virus
Mallard Ducks and Turkeys	Paramyxovirus type-2
Mallard Ducks and Turkeys	Paramyxovirus type-6

TABLE 2

HEMAGGLUTINATING VIRUS

ISOLATIONS FROM SENTINAL DUCKS

Location	No. Samples	No. Isolates	% Positive
Richmond	355	41	11.5
Paynesville	219	6	2.7
Hawick	320	25	7.8
Georgeville	262	14	5.2
Swift Falls	222	16	7.2
Willmar	233	35	15.0
1981 Totals*	1611	137	8.5
1980	656	109	16.6

*thru October 6

b. Field Outbreaks

In 1981 (till October) AI was reported in three turkey flocks in Minnesota. The first report was in breeder turkeys in the east central part of the state in June. A second outbreak occurred October, involving three market flocks located in Big Stone County (west central part of the state). At least two market flocks were involved in the third outbreak in central Minnesota. In all outbreaks the virus subtype was H5N2. A similar virus subtype was involved in market turkeys in North Dakota in July. The breeder turkeys experienced a marked drop in egg production (60 to 20%) with the usual problem with shell quality. No respiratory signs were seen in the breeders. The Big Stone County market flocks experienced severe respiratory distress and depression. The clinical signs were first noticed in the 16-week-old tom flock which spread to other two younger flocks (11 and 6 weeks). Influenza virus A infection with H5N2 subtype was confirmed serologically. Although efforts for virus isolation were made, no influenza virus was isolated. A domestic goose flock located a quarter mile from the farm was suspected to be the source of infection. A total 32,000 turkeys were involved in this outbreak with an average mortality rate of 20%.

The market flocks affected in central Minnesota experienced only mild clinical signs. The respiratory signs lasted only 3-4 days. The birds returned to normal health in a weeks time without experiencing any significant increase in mortality. An Influenza A virus subtype H5N2 was isolated both from tracheal and cloacal swabs. The source of this infection is unknown.

Research To Develop Improved Methods for Early Diagnosis

Preliminary trials are being done to standardize the enzyme-linked Immunosorbant assay (ELISA) test to assist in the early diagnosis of AIV infection. Sensitivity of the test and the short time required to conduct it makes it a valuable tool for early serodiagnosis of the infection. Sucrose and potassium tartrate glycerol gradients are used to prepare purified virus purification. Preliminary trials using conjugated rabbit antiturkey serum have given encouraging results. Currently, the double immuno diffusion (DID) test and Hemagglutination Inhibition (HI) tests are used for serodiagnosis.

Experimental Avian Influenza Vaccine Studies in Turkeys

The potency and efficacy of killed AIV vaccine was evaluated using 2, 4 and 6-week-old turkeys. The monovalent vaccine used in this study contained avian influenza virus strain H4N2 as a single antigen. Twenty birds in each age group received 1 ml. of the vaccine subcutaneously (first dose). Half of the vaccinated birds were given a booster dose (1 ml) four weeks later. The immune response of individual birds was evaluated using the DID and HI tests at weekly intervals.

The birds receiving a single dose of vaccine were challenged four weeks later. Those receiving two doses of vaccine, were challenged four weeks after the second dose. Each time a group of nonvaccinated/control birds were also challenged.

The A/turkey/Minn-Paynesville/80 (H4N2) virus was used as a challenge strain. The virus was administered intranasally. Three birds were sacrified from each challenge group for each age interval at three days post challenge. At necropsy two grams of lung, trachea and fecal material were collected separately for quantitative determination of their virus content. Tracheal and cloacal swabs were taken at the same time from the rest of the challenged birds for virus recovery. One week post-challenge all remaining birds were sacrificed and their air sac lesions scored for extent and severity of lesions. Some of the results of this experiment are given in Table 3.

RESULTS

1. The vaccine induced measurable antibody response both against the Nuecloprotein and Hemagglutinin antigens of the virus.

TABLE 3 - SEROLOGICAL RESPONSE FOLLOWING INFLUENZA VACCINATION

Group	Treatments (weeks of age) 1st dose vaccine	2nd dose vaccine	Live virus challenge	Weeks Post-Challenge 1 DID[a]	1 HI[b]	2 DID	2 HI	3 DID	3 HI	4 DID	4 HI	5 DID	5 HI	6 DID	6 HI	7 DID	7 HI	8 DID	8 HI	9 DID	9 HI	Virus Recovery 3 days Post-challenge No. of Samples Positive / No. of Samples Tested Cloaca	Trachea	Lung
I	2	none	6	0/10	0	1/10	0	2/10	2.6	2/8	1.10[c]	6/10	14/9	-	-	-	-	-	-	-	-	3/10	3/10	0/3
II	none	none	6	0/5	0	0/5	0	0/5	0	0/5	0[c]	2/5	2.0	-	-	-	-	-	-	-	-	4/5	5/5	3/3
III	2	6	10	0/10	0	6/10	0.16	8/10	4/6	4/10	4.6[d]	9/10	13.0	9/10	19.7	7/10	48.5	7/10	27.9[c]	7/10	256.0	3/10	0/10	0/3
IV	none	none	10	0/5	0	0/5	0	0/5	0	0/5	0	0/5	0	-	0	-	0		0[c]	2/2	64.0	3/5	4/5	3/3
V	4	none	3	1/10	0	5/10	0	5/10	8.6	2/10	8.6[c]	7/10	45.3	-	-	-	-		-	-	-	2/10	3/10	0/3
VI	none	none	8	0/5	0	0/5	0	0/5	0	0/5	0[c]	2/5	3.5	-	-	-	-		-	-	-	3/5	5/5	3/3
VII	4	8	12	0/10	0	2/10	0	9/10	0.20	7/10	4.3[d]	9/10	7.0	6/10	7.5	7/10	10.6	8/10	34.3[c]	7/10	831.7	1/10	1/10	1/3
VIII	none	none	12	0/5	0	0/5	0	0/5	0	0/5	0	1/5	2.0	0/5	no	0/5	1.5	0/5	0[c]	2/2	128.0	5/5	4/5	3/3
IX	none	none	10	1/10	0	2/10	0.15	6/10	3.7	5/10	7.0[c]	7/10	26.0	-	-	-	-	-	-	-	-	2/10	5/10	1/3
X	none	none	10	0/5	0	0/5	0	0/5	0	0/5	0[c]	2/5	3.0	-	-	-	-	-	-	-	-	2/5	5/5	3/3
XI	6	10	14	2/10	0	1/10	0.15	6/10	5.3	3/10	2.5[d]	10/10	7.0	8/10	18.4	8/10	64.0	9/10	36.8[c]	7/10	675.6	1/10	1/10	0/3
XII	none	none	14	0/5	0	0/5	0	0/5	0	0/5	0	1/5	1.7	-	0	-	0		0[c]	2/2	90.5	1/5	1/5	3/3

[a]Number birds positive/no. birds tested - double immunodiffusion

[b]Geometric Mean Titer - Hemagglutination inhibition

[c]Challenged with homologous strain

[d]Revaccinated

2. The vaccine did not produce any systemic or local adverse reactions.

3. The vaccine either completely prevented or significantly reduced viral replication in lung, trachea and the digestive tract.

CONCLUSIONS

The current epidemiological study suggests that the wild aquatic birds act as carriers of various influenza virus strains in their intestinal tract and excrete the virus in high concentrations. This serves as a potential danger to domestic poultry. A good influenza prevention and control program should address this problem. Complete confinement type of management would reduce the potential of infection from this source. Once the disease begins in an area, fast and quick diagnosis is essential. Isolation of the infected flocks from healthy flocks and vaccinating the susceptible turkey flocks with killed monovalent vaccine around an infected area, will help control the outbreak.

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TRANSPORT STRESS OF TURKEYS

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INTRODUCTION

Transport stress of turkeys is a condition characterized by leg myopathy and accumulation of subcutaneous edema. It has been called Turkey Leg Edema Syndrome, but such description is too narrow and focuses only on the edema accumulation. The condition has been observed in tom turkeys sporadically throughout the country, at least since the late 1960s. It is an economically significant problem when legs from such turkeys are condemned by the Inspection Service. Condemnation of one leg results in the loss of about 30% of the toms value because of the loss in grade as well as the lost weight.

MANIFESTATIONS OF TRANSPORT STRESS

Clinical observations are not helpful in detecting turkeys suffering from the condition. Observation of live birds at the farm or at the processing plant does not reveal any indication of a problem. So far no disease condition, nutritional practice or management procedure at the farm has been shown to contribute to the development of the condition in the turkey legs.

Post mortem examination of birds at the farm at the time of loading does not reveal the presence of subcutaneous edema or myopathy in the legs. No post mortem findings at the farm have been linked to the development of Transport Stress. Post mortem examination of turkeys after they have been on the truck for 14 hours, but before slaughter, reveals the presence of muscle lesions and edema in the legs. The edema fluid is usually clear to light yellow in color, but in occasional affected birds, it may be amber.

Lesions observed during post mortem of turkeys right off the truck do not differ significantly from lesions observed in the plant at the USDA inspection station. The exception is that in some birds at the plant, air gets into the inguinal space resulting in air bubbles or froth in the fluid.

Microscopic manifestations are acute hyaline degeneration of muscle fibers which is independent of the presence of edema; edema fluid which washes out during tissue processing, indicating it contains little or no protein; and alterations of the capillary endothelium. Inflammatory cells are generally absent. Interpretation of these microscopic lesions suggests:

1) Acute over exertion of muscles
2) Passive hyperemia (reduced flow of blood away from the leg)

*Department of Animal Science

Serum chemistry may become an aid in greater understanding of the condition. Early indications are that serum enzymes which indicate muscle damage are elevated in affected turkeys.

INCIDENCE

At the inspection station the condition is difficult to detect with accuracy. There is variation in condemnation rates between inspectors, plants, truck loads, farms, and days which makes research on the incidence of the condition quite difficult. This variation has also resulted in mistaken conclusions as to the cause and incidence of the condition. If the inguinal skin is incised then direct observation is possible, and an accurate measure of incidence and lesion score can be made. Using such a technique we recorded incidence and severity which is shown in Table 1. Inspection service trim for edema did not correlate with direct observation of the inguinal area.

In order to evaluate the effect of the surface of the processing truck, cage floor, comparisons were made of wood versus steel floors. The results are shown in Table 2. There may be an effect of cage floor on severity of the lesion.

This technique of actually observing the legs for the subcutaneous fluid is essential in researching the incidence and etiology of manifestations associated with transport stress of turkeys. Because the USDA inspector cannot see the fluid, he cannot be expected to detect it with accuracy. Consequently, inspection service data should not be expected to reflect the actual incidence of the condition.

Using the measurement technique of direct observation, continuing research will be directed at factors such as time and distance traveled, average body weight, loading methods, housing, season, environmental conditions and others.

TABLE 1

Average weight	No. checked	% affected	*Lesion score
25.1	52	48	1.1
27.0	49	14	2.8
26.4	150	18	1.1
24.2	200	9	1.1
23.2	100	4	1.0
25.8	300	18	1.3

TABLE 2

	% affected in lot no.			*Lesion score in lot no.		
	1	2	3	1	2	3
Wood floor	11	20	16	1.0	1.2	1.1
Steel floor	23	20	19	1.1	1.6	1.7

*Lesion score - birds were scored from 0 to 4 based on color and amount of fluid present. Average score of affected birds is shown.

PATHOGENICITY AND IMMUNITY STUDIES OF GAL E MUTANT OF *SALMONELLA TYPHIMURIUM* ON EXPERIMENTAL SALMONELLOSIS IN POULTRY

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INTRODUCTION

Bacteria of the genus *Salmonella* infect man and animals with the production of disorders which range from trivial to fulminating in their manifestations. In the United States Salmonellae are much more prevalent in fowl and swine than in other domestic animals. Considering the relative populations of these animals it seems that fowl are the greatest reserviors of the bacteria in this country. The extensive investigation carried with *Salmonella typhimurium* showed that the excretion of salmonella was early, regular and persistent. When young chicks were exposed to salmonella, a large portion became infected and shed the organisms in feces, without apparent ill effects.

Kumar *et al* (2) studied the sources of salmonella infections in nine turkey flocks and found the three most common sources to be the breeder flocks and hatcheries, the contaminated environment of the turkey houses, and contaminated feed.

Generally, antibiotics have not been extensively used in the treatment of salmonella infections of poultry because they have often proved less effective and more expensive than other medicinal treatment available. So salmonella infections, though frequent and world-wide in their distribution, are not adequately controlled by current preventive measures or available chemotherapeutic agents.

For many years, salmonellosis has been a disease of economic importance in poultry. Chicken flocks are commonly infected with salmonella, although infections are usually of little or no clinical significance. They do, at times, produce significant economic loss and usually pose a degree of public health hazard (4).

The study reported here concerns the establishment of intestinal infection, longevity of shed in the feces and antibody response as affected by vaccination with a live galactose epimerase mutant of *S. typhimurium*, strain G30D (gal E Mutant of *S. typhimurium*, strain G30D) and killed *S. typhimurium*, strain RW16. Since challenge infection itself constitutes a secondary stimulus in immunized animals, it was of interest to measure the antibody response to infection and persistence of shedding in animals immunized with live G30D or killed RW16.

MATERIALS AND METHODS

In Experiment 1, a preliminary experiment was performed in White Rock broiler chicks to determine the longevity of shed of G30D by testing cloacal swab cultures and the immune response by serological tests after oral inoculation with live G30D. Five groups of broiler chicks with 12 chicks in each group, were inoculated orally with G30D at different ages.

In Experiment 2, nine groups of female White Leghorn (WL) chicks with 8-10 chicks in each group, were inoculated with G30D by oral or subcutaneous inoculation varying from 0.28×10^8 to 2.8×10^8 cells. Two weeks and four weeks (oral vaccinated group) after vaccination those chicks were challenged orally with RW16.

In Experiment 3, four-week-old WL chicks (16 in each group) were used in this experiment. Two different vaccines, the live G30D and killed RW16, were administered orally and subcutaneously. All 11 groups of 4-week-old young chickens were given the first vaccination at 4 weeks and the second vaccination at 6 weeks of age. Two weeks after the second vaccination those chickens were challenged orally with RW16 along with the appropriate controls of nonvaccinated chicks.

The cloacal swab was taken once from each chick just prior to inoculation, each day in the first week, every other day in the second week, two days each week during the rest of the week post vaccination (PV) and post challenge (PC). The swabs were cultured in tetrathionate brilliant green broth (TBG Difco) and incubated at 42°C, 24 and 48 hours. Plating was made on brilliant green sulfapyridine agar (BGS Difco) or Salmonella-shigella +1% galactose agar (SSG Difco).

The sera were tested with non-motile S. typhimurium antigen by serum plate agglutination (SP) microtiter (MT) and microantiglobulin (MAG) tests are described by William and Whittemore (5,6,7). The significant titer were taken as 1:20 for the MT test and 1:40 for the MAG test.

The G30D mutant used as the live vaccine was kindly supplied by Dr. C. Wray, Central Veterinary Laboratory, M.A.F.F., Weybridge, England. The G30D strain showed typical biochemical and serological characters of S. typhimurium except that it did not ferment galactose. The RW16, used for the killed vaccine and as the challenged strain, was isolated from a field outbreak of salmonellosis in turkeys. The RW16 strain showed typical biochemical and serological characters of S. typhimurium except that it did not ferment inositol.

The one-way analysis of variance was used to test the significance of differences in the number of shedders and the differences in the number of reactors, between different groups of chickens.

The comparisons were made among means using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

All cloacal swab samples taken prior to inoculation were negative for salmonella. There were no clinical signs of salmonellosis detected at any time during the experiment. No uninoculated chicks yielded salmonella by culturing cloacal swabs or internal organs, and they were negative by serological tests. No G30D strain was detected by cloacal swab cultures or cultures of organ after oral challenge with RW16.

EXPERIMENTS 1 AND 2

The broiler chicks inoculated orally at one day of age started to shed the G30D on the first day PV; the highest numbers of shedders were detected during four to nine days PV and dropped sharply after 14 to 20 days (Table 1). No chicks showed positive serological test at the end of the experiment (Table 1). The broiler chicks inoculated orally at one week, two weeks, and four weeks of age shed G30D less than 14 days PV. Most of them shed G30D in the first seven to ten days PV and some chicks were positive on SP and MAG tests (Table 1).

This result indicated that G30D produced a relatively low agglutination response as determined by SP, MT and MAG tests. This result would indicate that if G30D has value as an immunizing agent, it would not interfere significantly with serological tests used in an official *S. typhimurium* program.

The chicks were inoculated with G30D orally and subcutaneously. Two groups (B.2, B.3, Table 2) of chicks inoculated subcutaneously died in 24 hours, and bacteriological cultures of heart, liver, spleen, and cecal junction were positive for G30D, but cloacal swab cultures were negative, and no gross lesions were detected. This result supported the finding of Pritchard *et al* (3). There were no deaths in the chicks inoculated subcutaneously with smaller doses and in a group which was inoculated orally with 9×10^8 cells of G30D (Table 2).

After chicks were challenged orally with RW16, there was a statistically significant difference in the numbers of shedders between the control and oral or subcutaneous vaccinated groups of chicks. Cloacal swab recoveries approaching 100% were noted in several groups of chicks early in the course of infection. Numbers of positive cloacal swab cultures dropped after 21 days (Table 3). It is logical to suggest that the level of salmonella excretion declined later in the course of infection. Therefore, cloacal swab cultures may not identify carriers that are not in the early active stage of infection.

There was considerable variation in the shed pattern of birds as indicated by recoveries from cloacal swab cultures. Some birds in untreated groups were persistently negative and others varied in their excretion rate. The results in this study have shown that chicks in vaccinated groups excreted the organisms consistently less than in the non-vaccinated group. This results corresponded to the work of Knivett and Stevens (1) and Pritchard *et al* (3).

In Experiment 3

The Effects of Live G30D and Killed RW16 Vaccines on the Immune Response of Young Chickens Before and After Challenge Orally with RW16.

The agglutination titers of the serum as determined by serological titers tests in groups that received the killed vaccine RW16 subcutaneously were much higher than the titers in the groups that were vaccinated orally or subcutaneously with the live G30D (Table 5).

The results have shown that the killed bacterin RW16 produced more reactors and high serological titer than the live vaccine (G30D). It is not known if this was due to the fact that it was more virulent and hence, produced a greater immunogenic stimulus or it might contain an immunogenic component not present in G30D. After oral challenge with RW16, the groups that received live G30D did not show higher titers than the groups receiving killed RW16. It seems that infection with G30D may be prevented by prior vaccination with a live vaccine.

The Effects of Live G30D and Killed RW16 Vaccines on the Shedders after Challenged orally with RW16.

After being challenged orally with RW16, the number of shedders in all nine vaccinated groups was found to be less than the numbers of shedders in the control group (Table 4). It was found also that there was a statistically significant difference in the numbers of shedders between control and nine vaccinated groups.

The double vaccination by different kinds of vaccines, live and killed vaccines, showed better results in the reduction of shedders than using the live or killed vaccine alone (Tables 4).

No statistically significant difference was found between the groups of chicks vaccinated by different routes (oral or subcutaneous) in the numbers of reactors or shedders.

From the practical point of view, the live vaccine might have considerable value in the control of S. typhimurium infection in the field. Vaccine G30D has three advantages. First, it did not produce high agglutination titers which could interfere with the serological tests used in the control program; secondly, the number of shedders of challenge strain RW16 were decreased in the group vaccinated with G30D; thirdly, chicks were not depressed for 24 hours after vaccination with live G30D as they were when vaccinated with killed RW16. It was also found out that the G30D strain could be considered to be a pathogen since the inoculation of large number (1.4×10^8 cells) of viable cells of G30D subcutaneously in one-day-old chicks did produce 100% mortality.

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Table 1. Numbers of shedders in White Rock Broiler Chicks Inoculated with Gal E Mutant of S. typhimurium, stain G30D.

Group of birds	Days postinoculation																		Serolog. tests 24 days PI		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20	24	SP	MT	MAG
A. 0	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12
A. 1	6/12	8/12	9/12	11/12	10/12	12/12	11/12	12/12	11/12	10/12	7/12	6/12	10/12	8/12	9/12	6/12	7/12	0/12	0/12	0/12	0/12
A. 2	0/12	4/12	3/12	4/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	*	*	*	*	*	2/12	0/12	2/12
A. 3	2/12	1/12	4/12	4/12	1/12	0/12	0/12	0/12	0/12	1/12	0/12	*	*	*	*	*	*	*	1/12	1/12	1/12
A. 4	6/12	0/12	2/12	2/12	3/12	1/12	1/12	0/12	2/12	1/12	1/12	0/12	*	*	*	*	*	*	2/12	2/12	3/12

A. 0 = Control
A. 1 = Inoculated orally one day of age
A. 2 = Inoculated orally one week of age
A. 3 = Inoculated orally two weeks of age
A. 4 = Inoculated orally four weeks of age

Numerator = No. of shedders
Denominator = No. of birds inoculated
* = Not determined
PI = Postinoculation

Table 2. Mortality of Experimental Groups of White Leghorn Chicks one day after Given Oral or Subcutaneous Inoculation with a live Gal E Mutant of S. typhimurium, strain G30D.

Groups of birds	No. of birds in a group	Inoculation				
		Age (days)	Inoc. (ml.)	No.of bacteria x 10^8	Route of inoc.	Live or dead
B. 1*	8	1	-	-	-	Live
B. 2	8	1	0.2	2.8	SC	All dead
B. 3	8	1	0.2	1.4	SC	All dead
B. 4	8	1	0.2	0.28	SC	Live
B. 5	8	1	0.2	0.14	SC	Live
B. 6	8	1	0.2	0.028	SC	Live
B. 7*	10	1	-	-	-	Live
B. 8	10	1	1.0	9.0	OR	Live

OR = Oral
SC = Subcutaneous
* Control groups

Table 3. Number of female White Leghorn chicks shedding S. typhimurium strain RW16 and reacting positively to the serological tests after challenge at two weeks of age (B.1, B.4, B.5, B.6) and four weeks of age (b.7, B.8).

Groups of birds	Days postchallenge																				Serolog. tests 52 days PC		
	1	2	3	4	5	6	7	8	9	11	13	15	18	21	26	29	32	37	42	47	52	SP	MT	MAG
B. 7	7/10	8/10	10/10	10/10	10/10	10/10	8/10	5/10	10/10	7/10	2/10	9/10	4/10	7/10	1/10	0/10	1/10	0/10	4/10	5/10	2/10	0/10	1/10	6/10
B. 8	9/10	8/10	10/10	10/10	8/10	4/10	4/10	4/10	1/10	2/10	1/10	2/10	1/10	0/10	1/10	0/10	0/10	2/10	4/10	3/10	2/10	0/10	0/10	3/10
B. 1	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	4/8	4/8	2/8	1/8	2/8	3/8	3/8	1/8	7/8	6/8	5/8	6/8	0/8	0/8	2/8
B. 4	7/8	8/8	7/8	8/8	8/8	7/8	7/8	6/8	7/8	2/8	1/8	4/8	1/8	2/8	0/8	0/8	6/8	1/8	0/8	1/8	0/8	0/8	0/8	2/8
B. 5	7/8	8/8	7/8	7/8	8/8	8/8	8/8	7/8	6/8	4/8	0/8	3/8	1/8	0/8	0/8	0/8	0/8	0/8	2/8	0/8	1/8	0/8	0/8	3/8
B. 6	8/8	8/8	8/8	8/8	5/8	2/8	3/8	2/8	2/8	1/8	3/8	2/8	0/8	3/8	3/8	2/8	1/8	6/8	0/8	0/8	0/8	0/8	0/8	2/8

B. 1 = Control
B. 4 = Inoculated subcutaneously 0.28×10^8 cells
B. 5 = Inoculated subcutaneously 0.14×10^8 cells
B. 6 = Inoculated subcutaneously 0.028×10^8 cells
PC = Postchallenge

B. 7 = Control
B. 8 = Inoculated orally
Numerator = No. of shedders or reactors
Denominator = No. of birds inoculated

Table 4. Number of female White Leghorn chicks, double vaccination with live or killed vaccines at four and six weeks of age, shedding S. typhimurium, strain RW16 after oral challenge at eight weeks of age.

1st vacc. 2nd vacc.	Days postchallenge														
	1	2	3	4	5	6	7	9	11	13	15	19	23	27	31
Con.chall.	16/16	15/16	15/16	15/16	14/16	15/16	13/16	11/16	10/16	10/16	3/16	3/16	2/16	2/16	1/16
SC L/SC L	10/16	5/16	5/16	12/16	9/16	4/16	8/16	6/16	6/16	3/16	0/16	2/16	3/16	1/16	2/16
SC K/SC K	11/16	8/16	6/16	13/16	14/16	8/16	7/16	2/16	6/16	1/16	3/16	3/16	2/16	3/16	4/16
SC K/SC L	10/16	10/16	0/16	6/16	3/16	2/16	2/16	4/16	3/16	2/16	1/16	3/16	1/16	4/16	3/16
SC L/SC K	10/16	6/16	9/16	6/16	8/16	5/16	5/16	1/16	1/16	4/16	1/16	1/16	0/16	0/16	0/16
SC L/OR L	12/16	8/16	12/16	12/16	9/16	9/16	11/16	2/16	6/16	8/16	2/16	3/16	1/16	2/16	1/16
SC K/OR L	13/16	7/16	6/16	13/16	10/16	8/16	5/16	6/16	5/16	2/16	0/16	0/16	1/16	0/16	0/16
OR L/SC L	11/16	8/16	14/16	15/16	8/16	9/16	6/16	1/16	4/16	1/16	2/16	2/16	2/16	1/16	0/16
OR L/OR L	16/16	5/16	12/16	8/16	11/16	1/16	4/16	3/16	5/16	3/16	3/16	1/16	1/16	1/16	0/16
OR L/SC K	15/16	4/16	5/16	6/16	8/16	2/16	2/16	4/16	5/16	2/16	0/16	2/16	1/16	1/16	0/16

OR = Oral vaccination
SC = Subcutaneous vaccination
L = Live vaccine
K = Killed vaccine
Numerator = No. of shedders
Denominator = No. of birds inoculated

Table 5. Experimental treatments of female White Leghorn chicks involved in numbers of chicks were positive in serological tests (reactor) in each bleeding after vaccination and after challenge.

Days	Control challenge			SC L/SC L			SC K/SC K			SC K/SC L			SC L/SC K			SC L/OR L			SC K/OR L			OR L/SC L			OR L/OR L			OR L/SC K		
	Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests			Serolog. tests		
	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG	SP	MT	MAG
13 after first vacc.	0/16	0/16	0/16	0/16	0/16	0/16	13/16	6/16	10/16	14/16	6/16	10/16	1/16	0/16	1/16	3/16	1/16	2/16	15/16	15/16	16/16	1/16	0/16	1/16	0/16	0/16	0/16	0/16	0/16	0/16
12 after second vacc.	0/16	0/16	0/16	0/16	0/16	0/16	11/16	7/16	5/16	7/16	8/16	5/16	9/16	6/16	4/16	0/16	0/16	0/16	4/16	1/16	4/16	0/16	0/16	3/16	0/16	1/16	1/16	9/16	8/16	8/16
8 after chall.	14/16	16/16	15/16	2/16	6/16	6/16	7/16	4/16	5/16	6/16	7/16	8/16	2/16	5/16	5/16	2/16	2/16	2/16	2/16	3/16	1/16	2/16	4/16	3/16	4/16	5/16	5/16	8/16	5/16	7/16
15 after chall.	13/16	14/16	15/16	4/16	2/16	6/16	8/16	6/16	10/16	6/16	4/16	7/16	4/16	7/16	11/16	1/16	2/16	3/16	7/16	2/16	4/16	1/16	1/16	5/16	4/16	2/16	7/16	10/16	5/16	7/16
28 after chall.	5/16	5/16	7/16	0/16	0/16	0/16	1/16	0/16	0/16	5/16	0/16	4/16	4/16	0/16	3/16	0/16	0/16	0/16	0/16	0/16	0/16	1/16	0/16	0/16	0/16	0/16	0/16	1/16	0/16	2/16

OR = Oral vaccination
SC = Subcutaneous vaccination
L = Live vaccine
K = Killed vaccine
Numerator = No. of reactors
Denominator = No. of birds inoculated

SIGNIFICANCE OF ENTERIC VIRUSES IN YOUNG TURKEY POULTS

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American Association of Avian Pathologists (AAAP) Committee on Nomenclature and Disease Reporting reported the occurrence of various avian diseases during the year 1979.[1]

The following TABLE summarizes the number of turkey flocks involved in enteric infections from the major turkey producing states.

State	Hemorrhagic Enteritis	Transmissible Enteritis	Unidentified Enteritis
North Carolina	65	21	64
Minnesota	75	--	--
California	74	2	178
Arkansas	16	90	16
Missouri	9	3	87
Virginia	4	--	8
Texas	3	--	9
Iowa	1	--	1
Indiana	6	1	2
Pennsylvania	12	--	27
Wisconsin	6	--	28
South Carolina	26	--	20
TOTAL	297	117	440
Percentage	34.8	13.7	51.5

Although the incidence of transmissible enteritis (also known as corona-viral enteritis and bluecomb) has decreased in recent years, unidentified enteric infections are on the increase accounting for 51.5% of the enteritis cases in 1979. This suggests the need for employing new techniques such as electron microscopy, virus isolation, immunofluorescence and enzyme-linked immunosorbent assays for rapid and specific diagnosis of unknown viral agents. In our laboratory, routine screening using electron microscopy and virus isolation on chicken embryo kidney cells showed that young turkey flocks were often infected with enteric viruses such as reo, rota, entero and paramyxo viruses.

Recently, 40 isolates of reovirus have been identified, 16 originating from Minnesota and 24 from other states. In 10 flocks, showing increased mortality and diarrhea, rotavirus was identified. In addition, entero-virus has been found in many of these outbreaks. Intestinal material from one of these outbreaks was used for a poult pathogenicity study. Forty percent mortality was observed on the first poult passage and 16.6% mortality on second poult passage. The weight gains of poults were depressed at 1, 2, 3, 4, 5, 6, 8 and 10 days post infection (DPI). Rotavirus and enterovirus were observed by election microscopy in fecal samples collected at 2 and 6 DPI. Survivor poults from poult passage 1

were challenge inoculated with turkey coronavirus (TCV) at 16 days. These poults were susceptible to TCV challenge becoming anorexic at 3 days and dehydrated at 5 and 7 days post challenge.

Bergeland et al also reported rotavirus infection in 10 flocks of turkey poults in South Dakota. Increased mortality occurred on the 13th day of age and continued for 5 or 6 days.[2]

Rotavirus infections have also been reported by Bartz and co-workers in Texas and they estimated the economic impact of the infection. In Farm A, 17,000 birds were housed and the total mortality reached 10% by 9 weeks. Total dollar loss on this farm was $18,500. Farm B having 8,000 birds suffered 40% mortality and a total dollar loss of $12,000 by the seventh week.[3]

Reoviral enteritis is also widespread in all turkey raising areas. Mortality of 3 to 10% has been associated with these outbreaks. The infected flock usually recovers after 4 to 5 days, although there is 10 to 15% unevenness in the flock. This growth retardation probably causes a greater economic loss than the actual mortality in the flock.

Prevention and control should be based on good management and sanitation since the continuous re-use of litter and brooding facilities by successive generations of turkey poults leads to an increased incidence of diarrhea. Disinfection, depopulation, thorough clean-up between flocks, traffic control and isolation brooding are necessary to break the disease cycle in successive flocks.

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EVALUATION OF THE NEWCASTLE DISEASE VACCINATION PROGRAMS OF TURKEYS

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INTRODUCTION

Newcastle disease virus (NDV) is a widespread and highly contagious viral pathogen that continues to have a profound economic impact on the turkey industry. Although various pathogenic forms of NDV exist around the world it appears that most NDV-related problems among Minnesota turkey flocks are caused by mild (lentogenic) endemic NDV strains that can be readily isolated from wild waterfowl and that apparently infect turkeys via their respiratory tracts.

Mild NDV strains may cause an inapparent infection that may have little or no economic consequence, but may impair a turkey's ability to protect itself against other infectious agents (i.e., E. coli) present in its environment. When NDV-related immunosuppression does occur in conjunction with faulty management practices, complicated NDV respiratory disease often results and is characterized in market turkey flocks by increased mortality (up to 30%) and poor flock performance (i.e., poor feed efficiency, big bird-little bird syndrome). Newcastle disease among turkey breeder flocks most often results in decreased egg production and poor egg shell quality.

Despite the widespread use of killed and live NDV vaccines by turkey producers, scientific evaluation of various turkey NDV vaccination programs has been limited and much of the information regarding NDV vaccination procedures (i.e., route, dose, vaccine strain) has been derived from studies involving chickens. Furthermore, the current standard method for evaluating NDV vaccines and/or vaccination programs consists of an intramuscular (IM) injection of velogenic NDV. Although the IM challenge method does evaluate the level of immunity associated with circulating serum NDV-antibody levels in the bloodstream (which are measured by the Hemagglutination-Inhibition (HI) test), it fails to measure the level of protection afforded the turkey by the local immune system within the respiratory system against NDV infection. Therefore, a research project was developed with the following objectives:

1. Develop an NDV aerosol challenge procedure by which to infect turkeys via the respiratory tract.

2. Evaluate various turkey NDV vaccination programs in terms of the level of resistance against NDV aerosol challenge.

3. Attempt to correlate serum NDV-antibody levels using HI titers with resistance to infection and/or disease by NDV aerosol challenge.

Experimental Method

Initial work on this project consisted of analyzing and evaluating a variety of factors pertaining to aerosol challenge, such as route of administration, selection of NDV challenge strain and dosage. After a series of preliminary experiments, the NDV challenge strain selected was a neurotropic Iowa field isolate identified as NDV 1519. A reproducible NDV aerosol challenge program was then developed in which field vaccinated turkeys are reunited with nonvaccinated flock mates (that are raised in isolation at the University of Minnesota from 1 day of age), weighed, bled and placed in isolation facilities. After a 24 hr. adjustment period, vaccinated and nonvaccinated birds are then subjected to NDV aerosol challenge at 2, 6, and 10 weeks post-vaccination (PV) as follows:

1. Oculonasal Challenge: Vaccinated and nonvaccinated turkeys in this group each receive 4 drops of a 1:100 dilution of NDV 1519. One drop (0.05 ml) is placed in each eye and nasal opening.

2. Spray Challenge: Vaccinated and nonvaccinated birds, housed in the same isolation room, are placed within a polyethylene tent and exposed for one hour to an aerosolized cloud of a 1:5 dilution of NDV 1519 that is generated by a Vineland Mister (M-1601) sprayer at various settings (depending on age of the birds).

3. Untreated Controls: Vaccinated and nonvaccinated turkeys are housed together but are not exposed to NDV.

Mortality and morbidity losses for each group of birds are recorded for 21 days post-infection (PI) and a sample of birds are bled at 14 and 21 days PI. Body weights of surviving birds in each group are recorded and compared.

Results and Discussion

Challenge results obtained thus far are as follows:

1. Farm A: A commercial flock of Nicholas male turkeys was spray vaccinated at 18 days of age with NDV (LaSota) vaccine (prepared as directed) and challenged as previously described.

Table 1: NDV Aerosol Challenge Results of Farm A Birds 2 Weeks PV.

Vaccinated	Method of Challenge	Avg. Gain in Body Weight (gms)/Bird	Mortality and Morbidity (%)	NDV GMT(HI Titers) Pre-	2 Week PC*	3 Week PC
No	Oculonasal	1432	50	0.0	≥512	53
Yes	Oculonasal	1629	0	8.6	36.8	8.0
No	Spray	1280	50	0.0	42.2	90.5
Yes	Spray	1431	0	18.4	21.1	10.6
No	Untreated	1726	0	0.0	0.0	0.0
Yes	Untreated	1731	0	8.6	10.6	0.7

*PC = Post-challenge

The above results (Table 1) suggest that the vaccinated birds were able to resist infection (no significant rise in HI titers) and disease (no mortality or morbidity, increased rate of gain) when compared to nonvaccinated birds, despite relatively low pre-challenge NDV GMT's.

Table 2: NDV Aerosol Challenge Results of Farm A Birds 6 Weeks PV.

Vaccinated	Method of Challenge	Avg. Gain in Body Weight (gms)/Bird	Mortality and Morbidity (%)	NDV GMT(HI Titers) Pre-	2 Week PC*	3 Week PC
No	Oculonasal	1768	67	0.0	90.5	≥90.5
Yes	Oculonasal	2121	0	2.6	52.0	19.7
No	Spray	1816	42	0.0	111.4	78.8
Yes	Spray	2267	0	4.0	111.4	128.0
No	Untreated	2169	0	0.0	0.0	0.0
Yes	Untreated	2346	-	2.1	0.0	2.0

*PC = Post-challenge

These results (Table 2) suggest that at 6 weeks PV, the vaccinated birds were able to resist disease (no mortality), but that the spray-challenged vaccinated birds may not have been as resistant to infection as the oculonasal-challenged vaccinated birds according to their respective serologic responses. Nevertheless, the difference in body weight gain/bird between the vaccinated and nonvaccinated birds in both challenged groups is impressive.

Table 3: NDV Aerosol Challenge Results of Farm A Birds 10 Weeks PV.

Vaccinated	Method of Challenge	Avg. Gain in Body Weight (gms)/Bird	Mortality and Morbidity (%)	NDV GMT(HI Titers) Pre-	2 Week PC*	3 Week PC
No	Oculonasal	1767	38	0.0	32.0	18.0
Yes	Oculonasal	1934	13	0.0	55.7	16.0
No	Spray	1253	13	0.0	111.4	32.0
Yes	Spray	1673	25	1.4	588.1	50.0
No	Untreated	1145	0	0.0	0.0	0.0
Yes	Untreated	1497	0	0.0	0.0	0.0

*PC = Post-challenge

These results (Table 3) indicated that at 10 weeks PV the level of resistance to infection and disease has diminshed for both groups of vaccinated birds that were challenged, particulary the spray-challenged vaccinates. These results suggest that an adequate level of resistance against NDV deteriorates between 6 and 10 weeks PV.

2. Farm B: A commercial flock of Nicholas hen turkeys was spray vaccinated at 21 days of age with NDV (LaSota) vaccine (prepared as directed) and challenged as previously described.

Table 4: NDV Aerosol Challenge Results of Farm B Birds 2 Weeks PV.

Vaccinated	Method of Challenge	Avg. Gain in Body Weight (gms)/Bird	Mortality and Morbidity (%)	NDV GMT(HI Titers) Pre-	2 Week PC*	3 Week PC
No	Oculonasal	840	58	0.0	$\geq$168.9	137.2
Yes	Oculonasal	1203	0	4.9	55.7	22.6
No	Spray	531	25	0.0	$\geq$181.0	84.4
Yes	Spray	861	0	9.8	39.4	19.7
No	Untreated	1013	0	0.0	0.0	0.0
Yes	Untreated	1092	0	11.3	22.6	9.8

*PC = Post-challenge

These results (Table 4) are similar to the results obtained for Farm A birds (Table 1) at 2 weeks PV. These results suggest that under the conditions of these experiments that NDV spray vaccinated birds had a high level of resistance to infection and disease as reflected by the mortality pattern, body weight gain and serologic response to challenge, despite relatively low pre-challenge NDV GMT's. It is of interest to note that several vaccinated birds during each of the above experiments had no detectable serum NDV-antibodies by the HI test yet survived challenge. Challenge results for Farm B birds at 6 and 10 weeks PV are not yet available.

CONCLUSIONS

1) Turkeys that are NDV spray vaccinated at 2 to 3 weeks of age do not develop high or persistant levels of circulating antibody.

2) The duration of protection against infection appears to be about 6 weeks.

3) The HI test results do not appear to correlate with protection in turkeys vaccinated at 2 to 3 weeks of age. In addition to preventing mortality, vaccination prevented weight losses associated with NDV infection.

ACKNOWLEDGEMENT

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SALMONELLA FEASIBILITY STUDIES IN TURKEYS

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The problem of salmonella infection in turkeys has been receiving special attention from the Minnesota Avian Disease Research group for several years. The research achievements have resulted in improved diagnostic aids for salmonella and modification of the Salmonella Control Program for Turkeys which is supervised by the Minnesota Board of Animal Health. Minnesota was the first state to receive the U.S. Pullorum-Typhoid Clean State classification under National Poultry Improvement Plan.

Despite the current control programs for pullorum-typhoid and typhimurium other salmonella and arizona serotypes are continually encountered in turkey breeding flocks and their progeny. In order to minimize the morbidity and mortality losses from salmonella and arizona infections the injection of day old poults with antibiotics at the hatchery is commonly practiced and the use of anti-salmonella and anti-arizona drugs in starter rations is a common practice. This results in an increased cost to the turkey industry of $3 to 5 million per year. In addition red meat and poultry are potential vehicles of salmonellas to humans and there is a public health concern.

Salmonella Feasibility Studies have been in progress at the College of Veterinary Medicine since 1978. These studies have been supported in part by a Cooperative Agreement with Veterinary Services - APHIS - USDA.

The three objectives of the project are being conducted in separate phases. They are as follows:

1. Determine whether it is possible under commerical conditions to maintain a breeder flock free of salmonella.

2. Determine whether the progeny from a salmonella free breeder flock be grown to maturity free of salmonella commercially.

3. Determine whether salmonella free progeny be produced from salmonella infected breeders.

PHASE I

A breeder operation and hatchery had been monitored extensively since 1972 for salmonella and arizona infections and was selected for this study. It was a closed operation except for the introduction of hatching eggs each year from the primary breeder for replacement breeders. The yearly operation involved approximately 7500 hens and approximately 600,000 poults were hatched over a six month period each year. The study was initiated in November, 1978 and the three year study was concluded in November, 1981.

Table 1 shows the number and kind of samples examined during the past three year period of intensive monitoring. Cultural examination of these samples did not reveal the presence of salmonella or arizona organisms in any of these samples. Blood samples taken during production period as well as at the end of the hatching season when the birds were marketed were also negative for seroconversion.

It was demonstrated from this three year study that a breeder flock free of salmonella and arizona organisms can be maintained under commercial conditions. Good managemental practices are the secret of this accomplishment. Important aspects of the management program in this breeder operation were:

1) Having salmonella free poults to begin with.
2) Farm environment free of salmonella and arizona organisms.
3) No animal by-products were included in the breeder candidate and breeder rations. Vegetable protein was substituted for animal by-products and fish solubles was used. All complete feeds were pelletized. There was full cooperation of the feed manufacturer.
4) Control of free flying birds and rodents on the farm.
5) Traffic control between farm buildings.
6) Evaluation of the program of salmonella control from time to time.

PHASE II

Two farms were involved in this study. Five flocks on one farm and two flocks on the second farm were identified for this study. Approximately 20,000 birds were involved in each flock. The source of the poults was the turkey operation mentioned in Phase I Study. The buildings involved in Phase II Study were cleaned and disinfected and after cleanup samples were examined for salmonella. Poults were placed in clean buildings and maintained on pelletized feed which contained animal by-products. Birds that died during the first 10 days of their life, dust and litter samples at intervals of 4, 8, 13, 16 and 20 weeks were cultured for salmonella.

TABLE 1

RESULTS OF BACTERIOLOGICAL EXAMINATION
OF SAMPLES FOR SALMONELLA/ARIZONA 1978-1981

SOURCE OF SAMPLE	NUMBER OF SAMPLES	RESULT
1. ENVIRONMENTAL		
a) DUST	3695	NO SALMONELLA/ARIZONA ISOLATED
b) LITTER	5226	" " " "
2. HATCHERY DEBRI		
a) INFERTILES	2764	" " " "
b) EARLY DEADS	2162	" " " "
c) DEAD IN SHELLS	2368	" " " "
d) PIPS	154	" " " "
e) FLUFF	192	" " " "
3. CLOACAL SWABS AT MIDPOINT OF LAY		
a) HENS	450	" " " "
b) TOMS	200	" " " "
4. FEED	171	" " " "
5. PROCESSING PLANT SAMPLES		
CECAL JUNCTIONS		
a) HENS (10% OF HENS PROCESSED)	1900	" " " "
b) TOMS (10% OF TOMS PROCESSED)	400	" " " "

When birds were marketed, cecal junctions were collected and examined from 10% of total birds processed.

The results were encouraging and also were disappointing on some occasions. Two turkey flocks remained free and went to market free where the farm environment was maintained free of salmonella. Birds reared on the farm that had multiple age flocks and other buildings of the farm that had turkeys with salmonella, became infected with the salmonella which was already existing on the farm. The encouraging results indicated that birds from a salmonella free source can be reared free of salmonella under commercial conditions to maturity when the farm environment was maintained free.

PHASE III

This is the most important among the three phases. Studies are in progress to determine whether we can prevent salmonella from cycling in the infected breeder flock and salmonella free progeny can be produced from salmonella infected breeders. Vaccination of infected breeder flock is being presently attempted to see whether transovarian transmission can be prevented and reduction of intestinal shedders may be obtained. Encouraging results have been obtained under laboratory conditions in reducing shedding of infected birds. The application of this knowledge under field conditions is being planned.

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** Irvin Peterson - Coordinator of the Feasibility Studies, Veterinary Services, APHIS-USDA, Federal Building Hyattsville, MD 20782

INHALATION OF PATHOGENS - AN APPARATUS FOR MEASURING HOW MANY

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Abstract

Beerwinkle and Oehler (1979) presented a nose only exposure system. Their system used concurrent flow spirometry to furnish the respiratory volume and rate measurements needed to evaluate animal response to known quantities of solid or liquid aerosols. At the University of Minnesota we have taken Beerwinkle's design and modified it to decrease losses and determine aerosol viability along with size and quantity.

General Description

The animal respiratory challenge unit we are constructing and testing is designed to deliver a known quantity and size distribution of a bacterial aerosol to a mask or chamber and determine the amount expired. The key components of the system are:

- a nebulizer to create the bacterial aerosol
- impactors to analyze the delivered and expired aerosol streams
- concurrent flow spirometer for real time respiratory volume and rates.

A schematic of the entire unit is shown in Figure 10.

The challenge system is suitable for animals with up to 30 L/min respiratory rates limited primarily by the spirometer. As shown in Figure 10, a bacterial aerosol is created from a concentrated bacterial solution through a compressed air nebulizer. The bacterial solution will be supplied to the nebulizer by a syringe pump at such a rate to starve the nebulizer supply. After initial start-up the nebulizer will come to equilibrium at the net flow conditions and what is fed to it will be aerosolized as long as the feed rate does not exceed the supply needed.

The aerosol stream will be mixed with a humidified stream of sheath air. The mixed air stream will be run through a charge neutralizer to reduce the average static charge on the aerosols (Liu and Pui, 1974). This reduces the losses to the chamber and tube walls. The radioactive source we have selected, polonium 210, is locally supplied and disposed of by Minnesota, Mining and Manufacturing at about a tenth of the cost of the more common Krypton 85 source.

The polonium 210 has a useful life of about one year versus ten years for Krypton 85, and is safe to handle unlike Krypton 85.

The neutralized, mixed aerosol stream empties into a large chamber with a volume about three to four times the respiratory rate for the test animal. The temperature and relative humidity of the mixture are measured. A sample of the mixture is continuously run through an impactor for size, quantity and viability measurements. The respiratory air required by the animal is drawn out of the chamber through a one way valve. The remaining aerosol is exhausted through an absolute filter. The expired air of the animal enters an exhaust chamber. The contents of this chamber are also sampled continuously for aerosol description. The walls of the exhaust lines and chambers will be heated to eliminate condensation losses of the humid expired aerosol. The exhaled air exits into the exhaust stream of the initial mixing chamber. A 13.5-L spirometer is connected to the exhaust stream through a 3-way valve. The valve allows the system to be vented to the room by to bypass the respirator The concurrent flow spirometer is sensitive to pressure changes in the exhaust stream of the closed loop aerosol system.

The major modification to the Beerwinkle device is to the cascade impactor design. Beerwinkle's impactor gave mass versus particle diameter range. This was correlated to number versus diameter range. The total viability of a bacterial stream was tested from an absolute filter. Our impactor, Figure 9, can run at a variety of flow rates and determines both mass and viability versus particle diameter ranges. The design of our cascade impactor follows the procedures developed by the University of Minnesota particle technology lab and reported by Marple and Willeke (1976).

The procedure is briefly to follow a flow rate and cut sizes. Through manipulation of Stokes equation and Reynolds number we have

$$Q = \frac{\pi}{12} \left(\frac{\zeta \rho}{Stk_{50}}\right)^{1/2} \left(\frac{Re}{\zeta}\right)^{3/2} y\sqrt{c}\, D_{50} n \qquad \text{Equation 1}$$

where:

Q = flow rate

ζ_ρ = particle density

Stk_{50} = stokes number at 50% cutoff

Re = Reynolds number

ζ = fluid density

y = fluid viscosity

C = Cunningham's slip coefficient

D_{50} = cut size

n = number of jets

The nature of the stokes number is such that for round impactor jets there is little variation for a range of Reynolds numbers of 100 to 20000. The stokes number is also stable for increasing jet to plate distances. Marple and Willeke recommend a S/W of 1 where 5 is jet to plate distance and W is jet diameter; a T/W of 1 where T is throat length; and a Reynolds number of 3000.

With this information we have developed a family of design graphs to aid in selection of the various stages of the impactor. The graphs are a plot of iterative solutions of equation 1 and give jet diameter for a given Reynolds number cut size and stokes number. An additional family of curves gives the number of jets required for a given flow rate and cut size. Several of these plots are shown in Figures 1 - 8. The iterative procedure is stored on an Apple II computer disk to allow later determination of cut sizes for the actual experimental flow rate used.

To allow collection of bacteria and later determination of viability, we are using sterile filter paper disks coated with a 3 per cent agar solution. This agar solution does not completely gel to allow a viscous surface to catch the impacted bacteria. After the test the filter paper and wating can be removed and immersed in a growth solution that can be serially diluted. The serial dilution can quantify the number of viable bacteria above given cut size. Selective growth media can be used to isolate particular bacteria under examination.

In view of the bacterial experiments under consideration the impactor is constructed from aluminum and stainless steel for autoclaving resistance. Also, the design is such that we can have a stock of different stages or cut sizes to configure many different cascade impactors for particular purposes. With the backup software, a variety of flow rates can also be used and the exact impactor cut sizes determined.

Upon completion of the challenge apparatus, it will be tested to obtain a family of calibration curves defining system losses versus flow rate and aerosol concentrations. This will be accomplished using a radio tagged bacteria strain, without an animal in the system. Also chamber or mask losses will be identified.

References

Beerwinkle, K. R. and Dehler, D. D. 1979. A nose only inhalation exposure system for large animals. ASAE paper #79-5518. Presented at the American Society of Agricultural Engineers 1979 winter meeting in New Orleans.

Liu, B. Y. H. and Pui, D. Y. H. 1974. Electrical neutralization of aerosol. J. Aerosol Sci. 5:465-472.

Table 1. Component List.

C_{1-2}	One way valves
EC	Exhaust chamber
FF	Filter
F_{1-4}	Filters
G_{1-9}	Pressure gauges
H	Humidifier
IC	Inhalation mixing chamber
I_{1-2}	Cascade impactor
K	Charge neutralizer
M_{1-7}	Flow meters
MT	Moisture trap
N	Compressed air nebulizer
RH	Temperature and humidity measurements
R_{1-2}	Regulators
S	Syringe pump
Sp	Spirometer
V_{1-10}	Valves

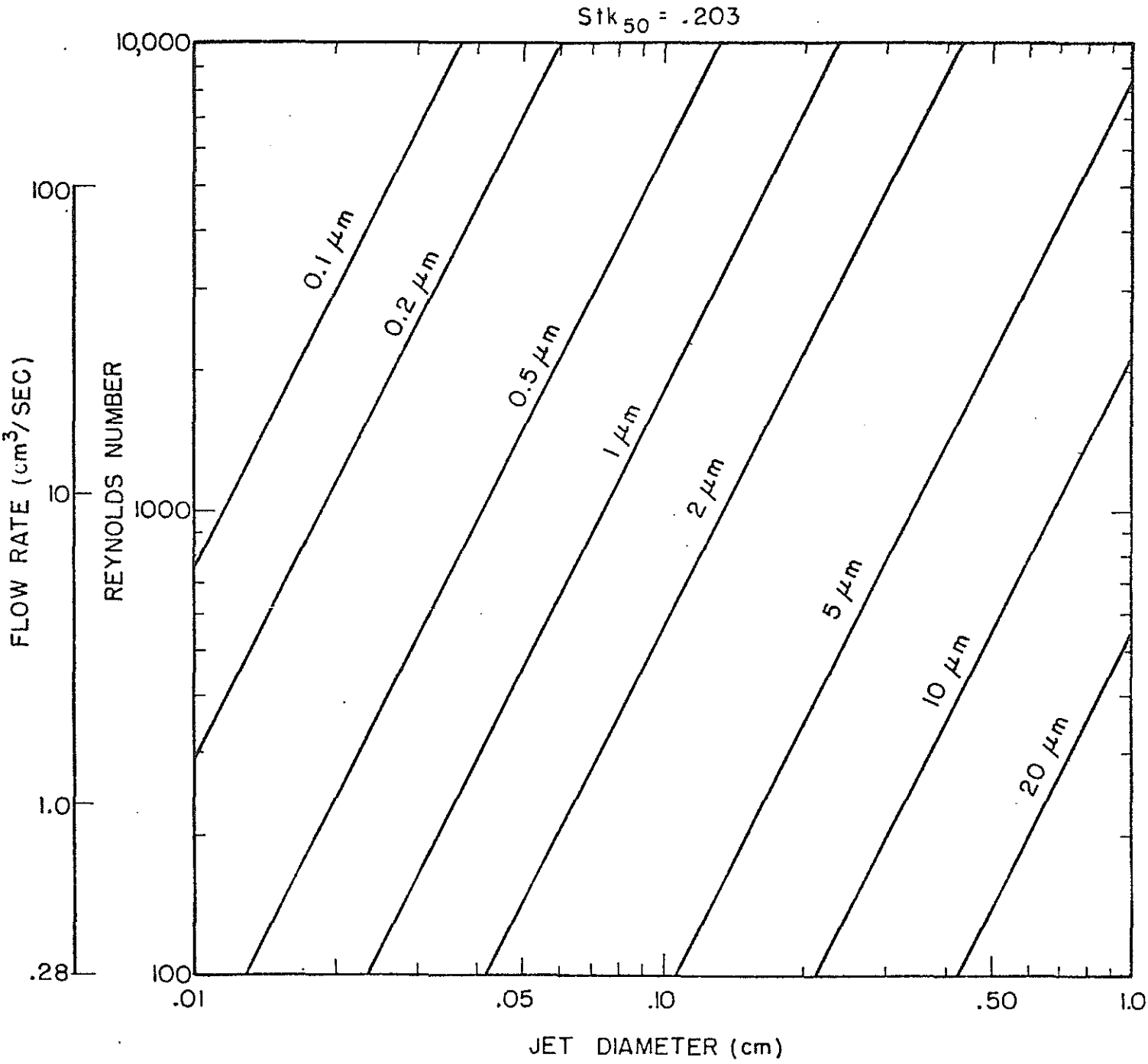

Figure 1. Design chart for jet diameter given aerodynamic particle cut size, flow rate and Stk_{50} = .203

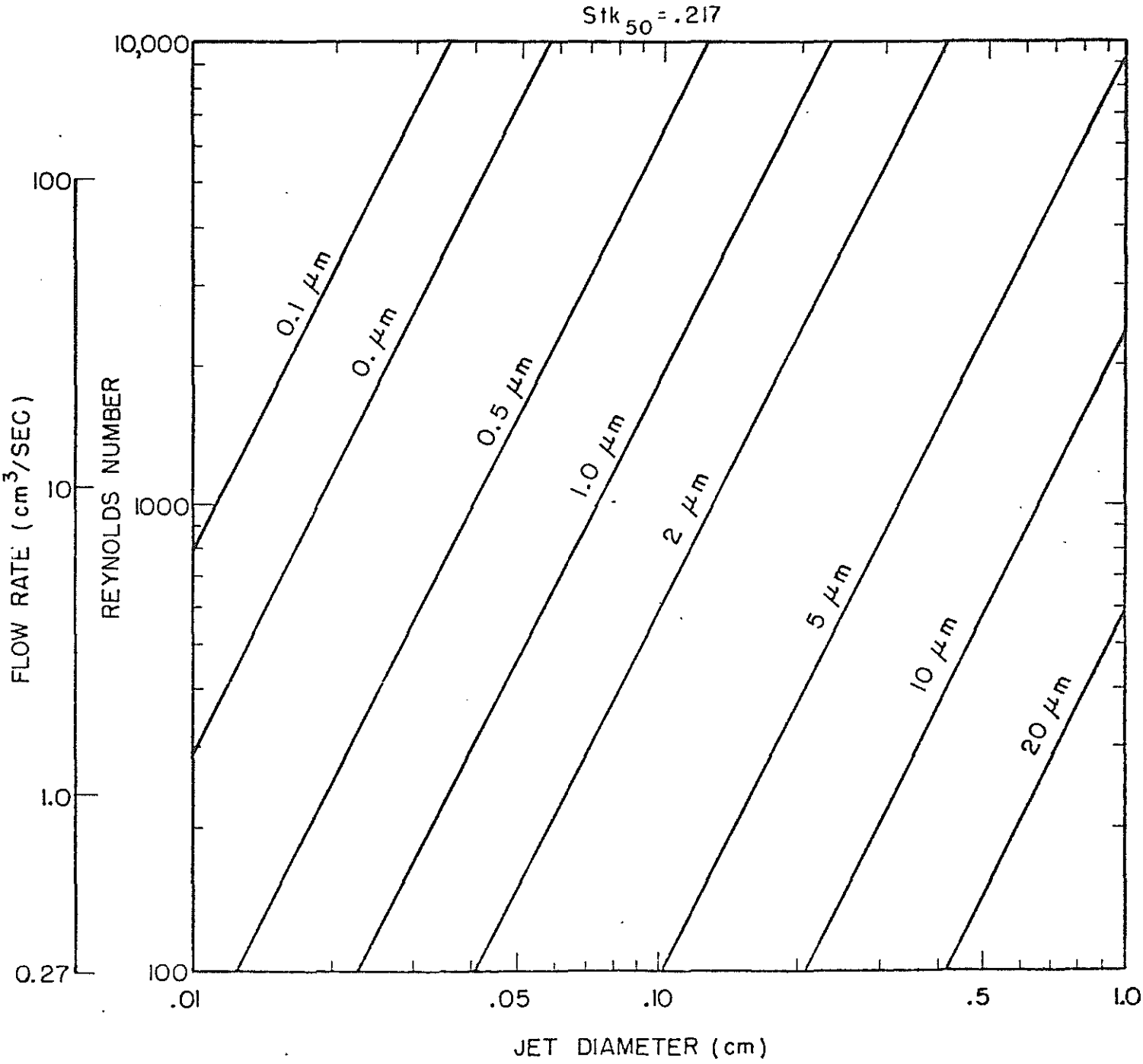

Figure 2. Design chart for jet diameter given aerodynamic particle cut size, flow rate and Stk_{50} = .217

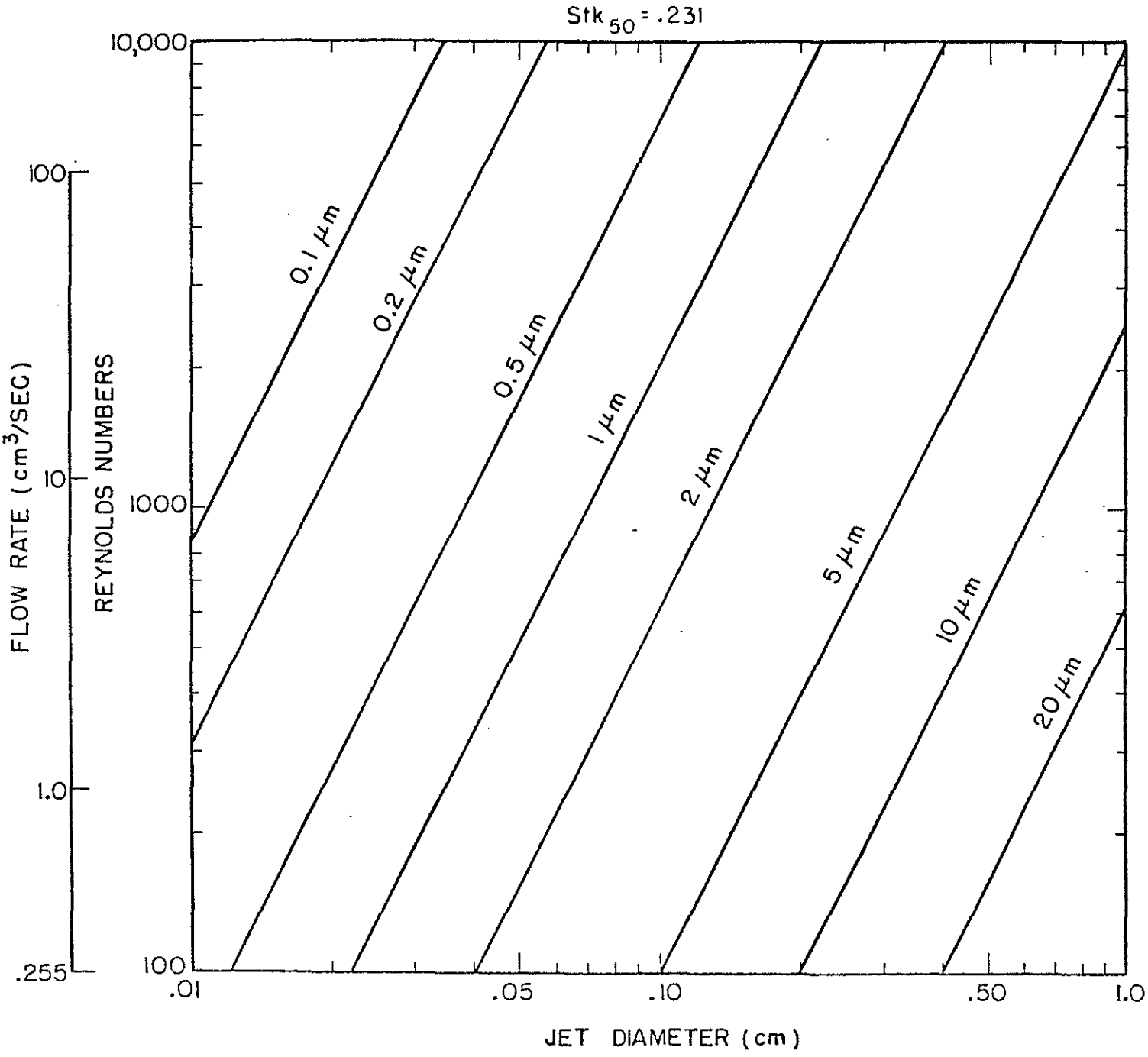

Figure 3. Design chart for jet diameter given aerodynamic particle cut size, flow rate and Stk_{50} = .231

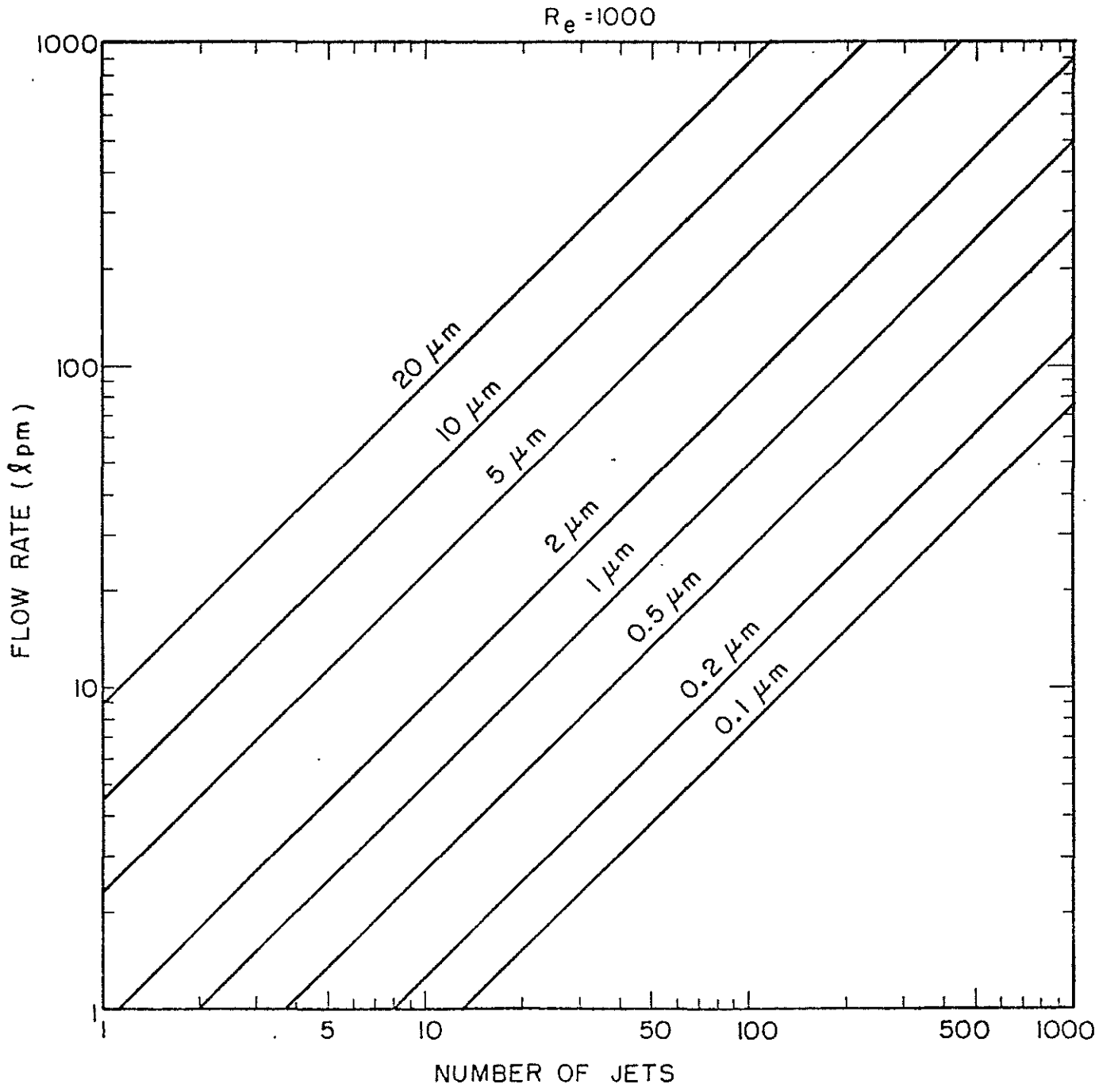

Figure 4. Design chart for number of jets required for a given flow rate, Reynolds number and cut size.

Re = 1000

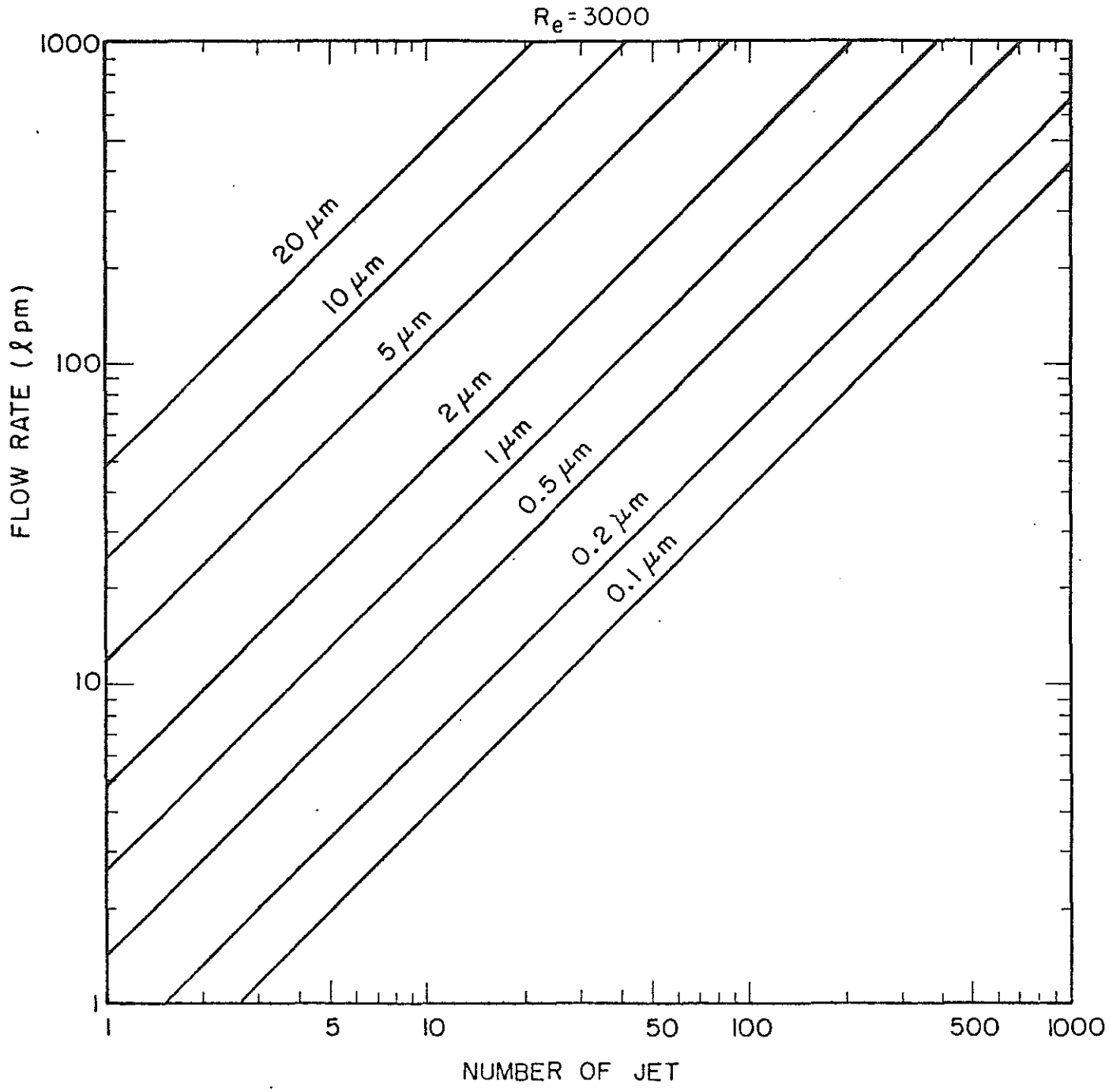

Figure 5. Design chart for number of jets required for a given flow rate, Reynolds number and cut size.

Re = 3000

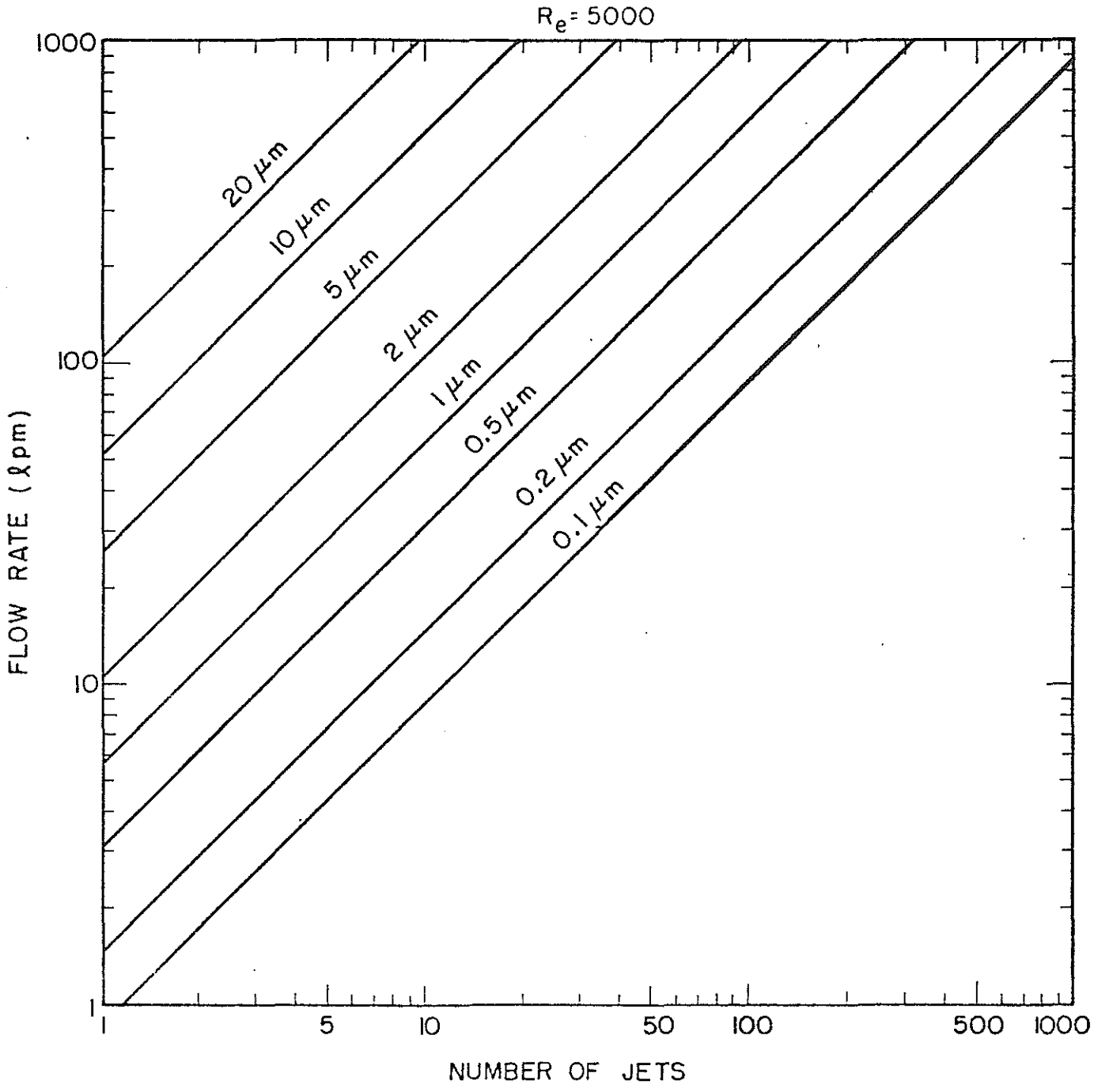

Figure 6. Design chart for number of jets required for a given flow rate, Reynolds number and cut size.

Re = 5000

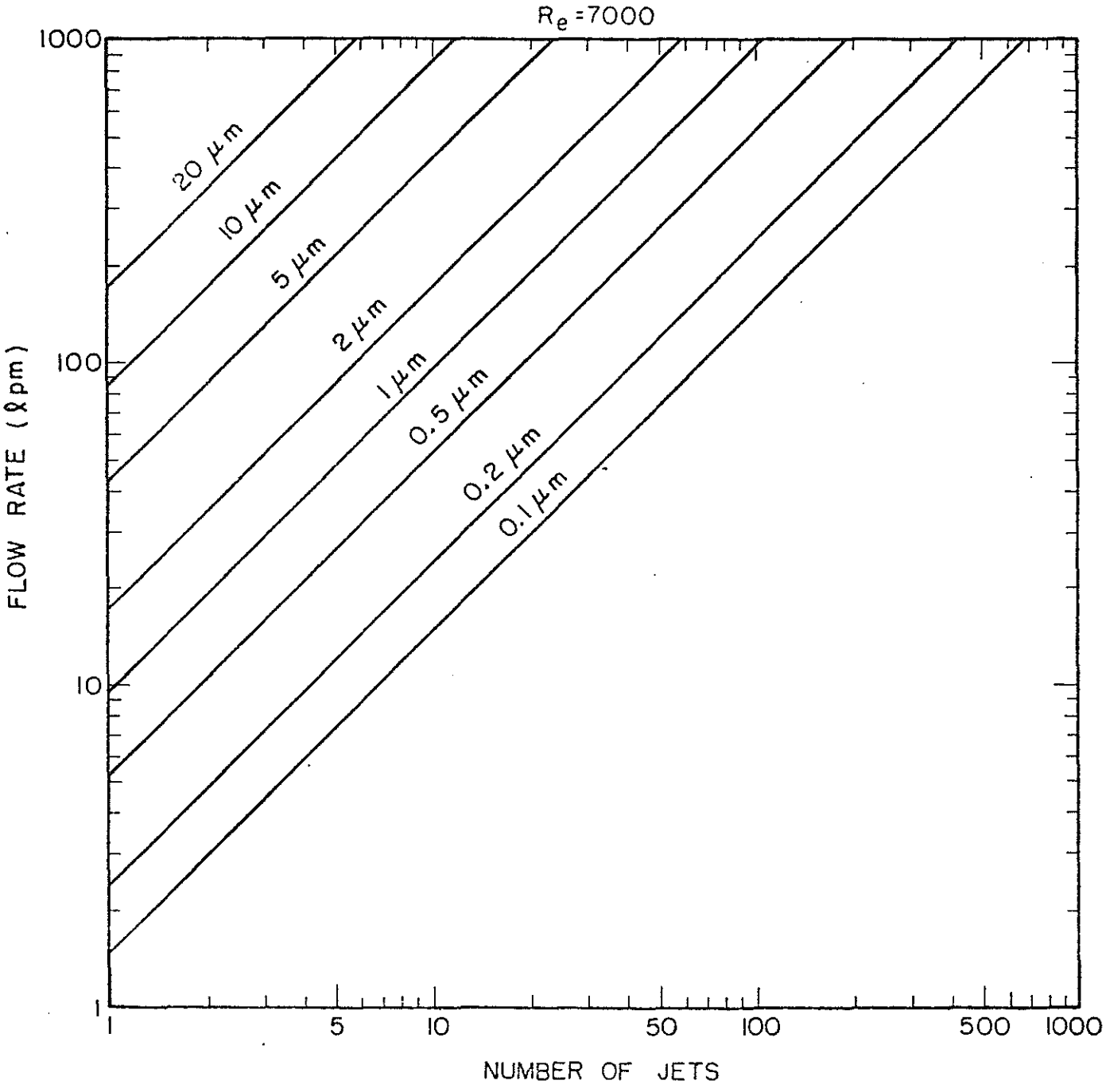

Figure 7. Design chart for number of jets required for a given flow rate, Reynolds number and cut size.

Re = 7000

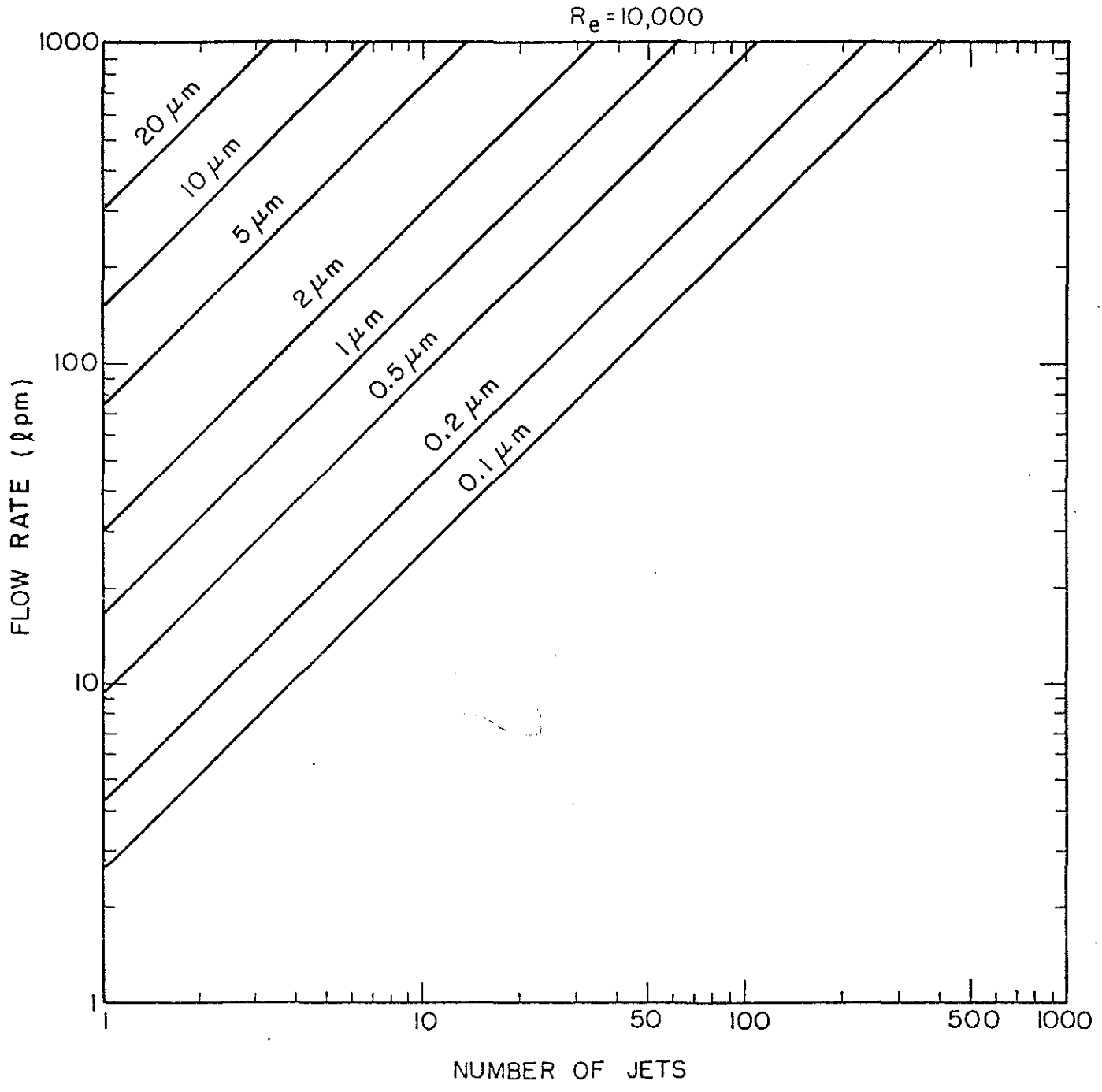

Figure 8. Design chart for number of jets required for a given flow rate, Reynolds number and cut size.

R_e = 10000

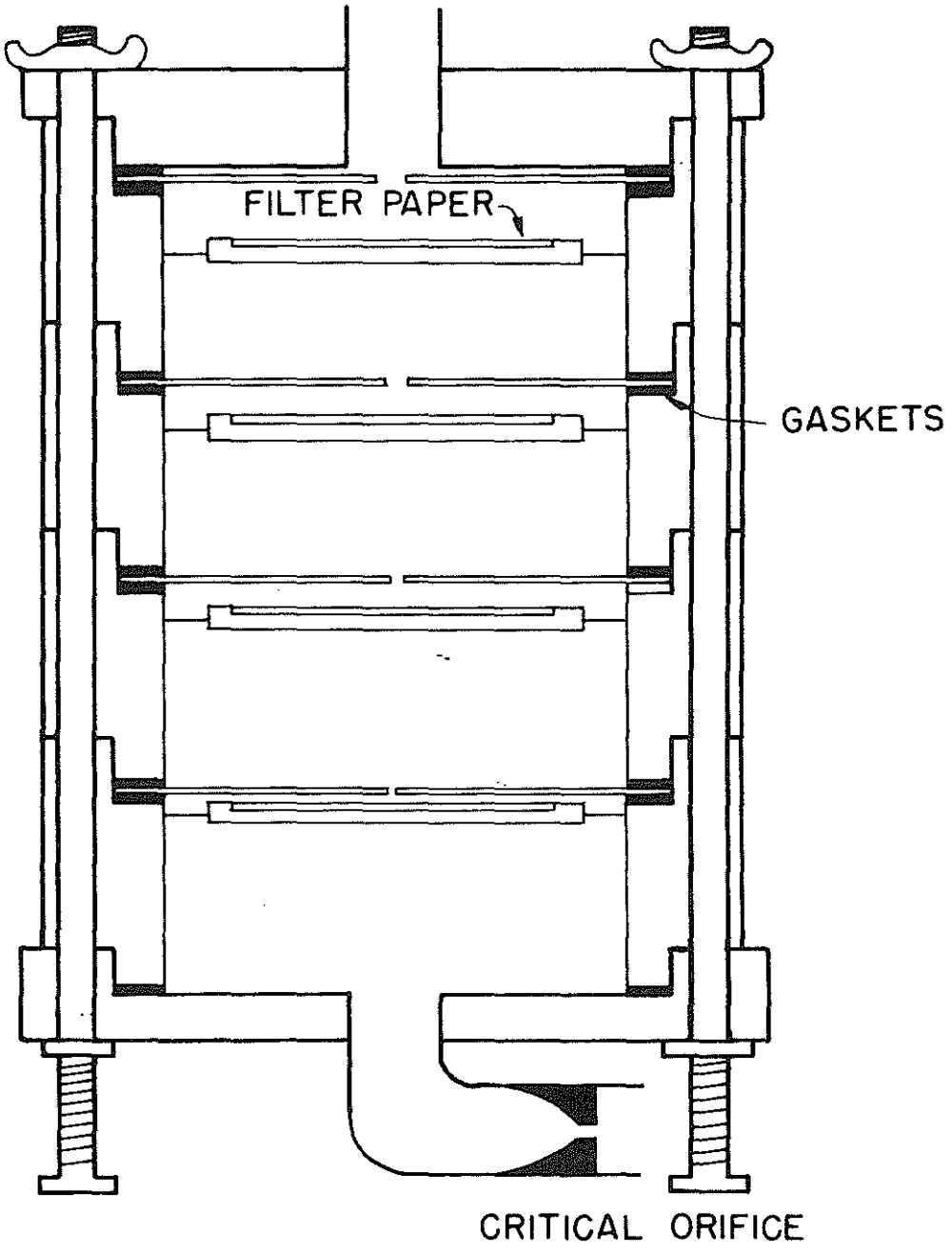

FIGURE 9. CASCADE IMPACTOR

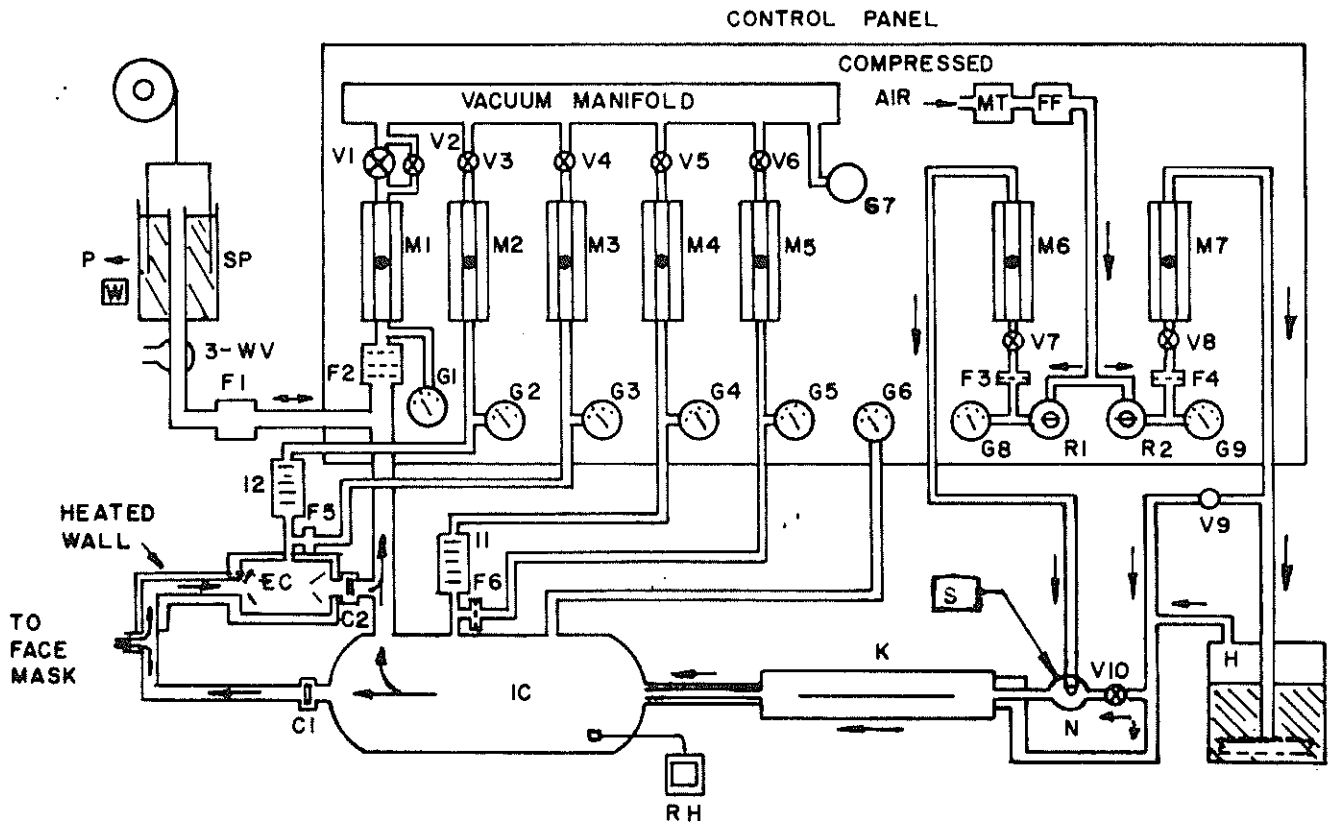

FIG. 10. SCHEMATIC DIAGRAM OF COMPLETE INHALATION EXPOSURE SYSTEM. COMPONENT IDENTIFICATION LISTED IN TABLE 1.

HERITABILITY OF CONGESTIVE CARDIOMYOPATHY IN THE TURKEY

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Congestive cardiomyopathy (CM) in the domestic turkey affects the young poult with peak death losses at two to five weeks of age. The disease was first described by Magwood and Bray (Canad. J. Comp. Med., 1962). They established an inbred strain with a high incidence of the disease.

Gross dilation of either or both ventricles occurs and is the characteristic lesion on autopsy. Endocardial fibroelastosis is noted by light microscopy. Swollen mitochondria containing dense granules tentatively identified as calcium phosphate crystals, and C-type virus-like particles in the sarcoplasmic reticulum measuring 60-90 mμ diameter are demonstrated in EM sections (Staley, N.A. et al., Am. J. Vet. Res., 1972). In addition to possible viral etiology, glycogen storage disease (Czarnecki, C.M. et al., Avian Dis., 1975), alpha-1-antitrypsin deficiency (Rattner, D., J. Comp. Path., 1976), immune mediated CM (Staley, N.A. et al., Cardiovas. Res., 1981), environmental factors such as Furazolidone toxicity (Jankus, E.F. et al., Avian Dis., 1972) and genetic causes have been suggested or identified as contributing to the incidence of the disease.

This genetic study was initiated in 1972 to investigate the heritability of turkey CM using the strain established by Magwood and Bray.

HYPOTHESIS

Congestive cardiomyopathy of the domestic turkey is inherited.

EXPERIMENTAL DESIGN

1. Correlation of electrocardiographic (ECG) data with CM lesions.

    a. ECG data gathering at 3 and 5 weeks of age.
    b. Autopsy poults at 5 weeks of age and subjectively score each ventricle for dilation on scale of zero (no dilation) to 4 (maximum dilation). Thus, total heart score range zero to 8.
    c. Calculate ratio of heart ventricles to body weight.

2. Heritability (h^2).

    a. Pedigree breeder hens and toms using artificial insemination and cage confinement of laying hens.
    b. Use nested model analysis of pedigree data based upon a metric ECG measure to calculate h^2 after method of Falconer (Introduction to Quantitative Genetics, The Ronald Press, 1960).

RESULTS

Twenty-one percent of the 758 cases used for correlation of ECG data with CM lesions had CM (Table 1). The incidence of males with CM was about twice that of females, 103 vs 56 (Table 1).

Utilizing a standard discriminant analysis program (SPSS, Mc Graw-Hill, 1975), the F to Enter and Raos V statistics were calculated from ECG and related measured parameters (Table 2). It is to be noted that the lead aVF R wave amplitude at 5 weeks (FR2) was the most discriminating function to separate the CM poult from a normal one.

The distribution of lead aVF R wave amplitudes by total heart score is illustrated in Figure 1. The correlation coefficient of thes distribution is 0.89. A correlation coefficient of 0.86 is calculated from the distribution of lead aVF R wave amplitude by ventricular to body weight ratio (Figure 2).

Data in Table 3 is derived from the assumption that lead aVF R wave amplitude greater than 3 mm (0.15 mv) defines a CM poult. This single ECG metric value correctly diagnosed 90.5% of the 758 cases (Table 3). Based on lead aVF R wave amplitude, 29.4% of the cases are CM compared to 21% by ventricular dilation. Applying the R wave amplitude assumption significant differences ($p<0.001$) in total heart scores (2.06 vs 0.02) and ventricular to body weight ratios (6.6 vs 4.3) exist between CM and normal poults (Table 4).

Utilizing the metric measure of lead aVF R wave amplitude the h^2 value for both sexes combined is 0.26 (Table 5). The female heritability is greater than the male (0.49 vs 0.17) and the female phenotypic variance (V_p) is less (9.4 vs 22.4) (Table 5).

DISCUSSION

The correlation of lead aVF R wave amplitude at 5 weeks of age with ventricular dilation (0.89) and ventricular to body weight ratios (0.86) is remarkably good. The subjective scoring of ventricular dilation was conservative as illustrated by only three cases of CM (heart score of 1 or more) with a lead aVF R wave amplitude less than 3 mm (0.15 mv) (Figure 1). The lead aVF R wave amplitude of 3 mm or greater at 5 weeks of age is a good predictor of CM as demonstrated by correctly diagnosing 90.5% of the 758 cases studied (Table 3). The significant differences ($p<0.001$) in ventricular to body weight ratios (6.6 and 4.3 grams Kg^{-1}) for CM and normal poults support the validity of this metric measure as a diagnostic tool.

A sex dimorphism exists in the distribution of CM with males having about twice the incidence of females, 103 vs 56 (Table 1). This

dimorphism is also apparent in the phenotypic variance (V_p) which is less for females (9.4) than males (22.4) (Table 5). This sex dimorphism associated with CM is unexplained. Generally, a sex dimorphism is not recognized in the turkey until about 8 weeks of age when weight differences are expressed.

The following is an example of the use of the h^2 coefficient.

progeny mean = h^2(mid-parent value — population mean) + population mean

Assumptions:

1. Population mean is 1 mm (0.05 mv).
2. Select mid-parent value of 9 mm (0.45 mv).

Therefore;

progeny mean = 0.26(0.45 — 0.05) + 0.05

progeny mean = 0.15 mv

If the progeny median were also 0.15 mv, more than 50% of the poults from this mating would have CM.

CONCLUSIONS

1. Congestive cardiomyopathy in the domestic turkey is inherited.

2. A heritability coefficient of 0.26 may be used to estimate breeding value for congestive cardiomyopathy when applied to lead aVF R wave amplitude data measured at 5 weeks of age.

Table 1: Distribution of 758 cases by diagnosis and sex.

Diagnosis	Sex		
	Female	Male	Row total
Cardiomyopathy	56	103	159 (21%)
Normal	344	255	599 (79%)
Column total	400(53%)	358(47%)	758 (100%)

Table 2: Discriminant analysis of ECG and measure parameters to separate cardiomyopathic from normal poults.

Variable	F to enter	Raos V	Change in Raos V	Significance (ρ)
FR2*	1415	1415	1415	< 0.001
FR1**	219	2047	632	< 0.001
FT2	79	2343	296	< 0.001
TR2***	12	2393	49	< 0.001
FST	4	2409	17	< 0.001
Heart rate	3	2422	13	< 0.001
Weight 5 weeks	4	2437	15	< 0.001
FS2	4	2452	15	< 0.001
FS1	3	2465	12	< 0.001
TS1	4	2481	16	< 0.001
FT1	1	2485	4	0.040
TR1	1	2490	5	0.028

* 5 week R wave lead aVR

** 5 week R wave lead I

*** 3 week R wave lead aVR

Table 3: Predicted diagnosis based on lead aVF R wave amplitude at 5 weeks.

Actual diagnosis based on total heart score		Predicted* Normal**	Cardiomyopathy#
Cardiomyopathy	159 (21.0%)	4	155
Normal	599 (79.0%)	531	68
Column totals	758	535 (70.6%)	223 (29.4%)

* 90.5% of diagnosis based on total heart score >1(equivalent to cardiomyopathy) correctly diagnosed on basis of lead aVF R wave amplitude.

** Lead aVF R wave amplitude <0.10 mv.

Lead aVF R wave amplitude >0.15 mv.

Table 4: Predicted diagnosis based on lead aVF R wave amplitude at 5 weeks.

Diagnosis	N of cases	Total heart score Mean±s.e.	Ventricular to body weight ratio Kg^{-1} Mean±s.e.
Cardiomyopathy (R>0.15mv)	223	2.06±0.125*	6.6±0.012*
Normal (R<0.10mv)	535	0.02±0.012	4.3±0.002

* $\rho<0.001$

Table 5:Heritability of turkey cardiomyopathy based on R wave amplitude of lead aVF at 5 weeks (1972 thru 1979).

Sex	N	h^2±s.e.	Significance	V_p*
Females	752	0.49±0.18	$\rho<0.01$	9.4
Males	673	0.17±0.20	NS	22.4
Both sex#	1479	0.26±0.11	$\rho<0.05$	15.6

* Phenotypic variance
# Includes 54 undetermined sex cases

Figure 1: Distribution of lead aVF R wave amplitudes by total heart scores.

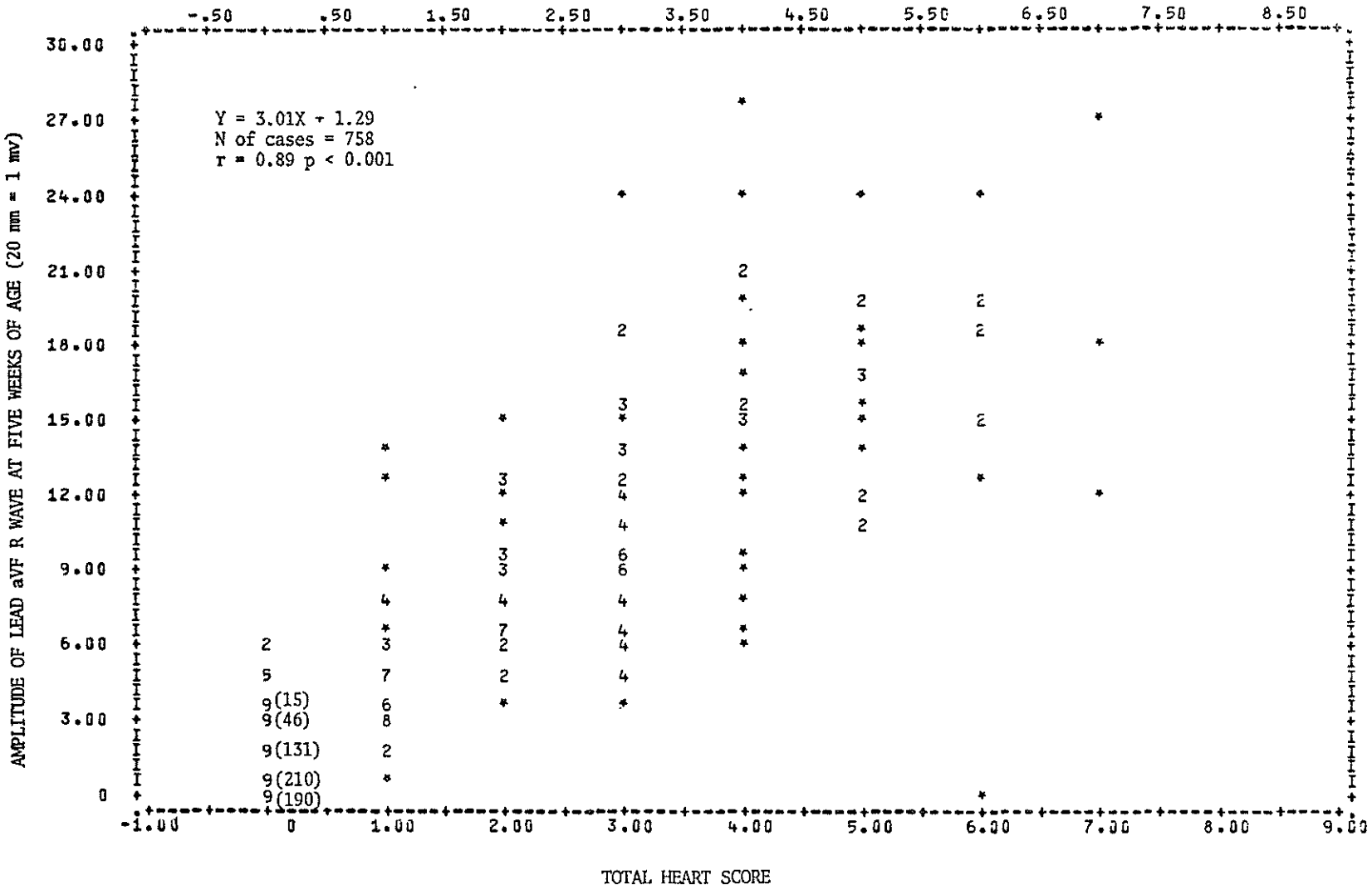
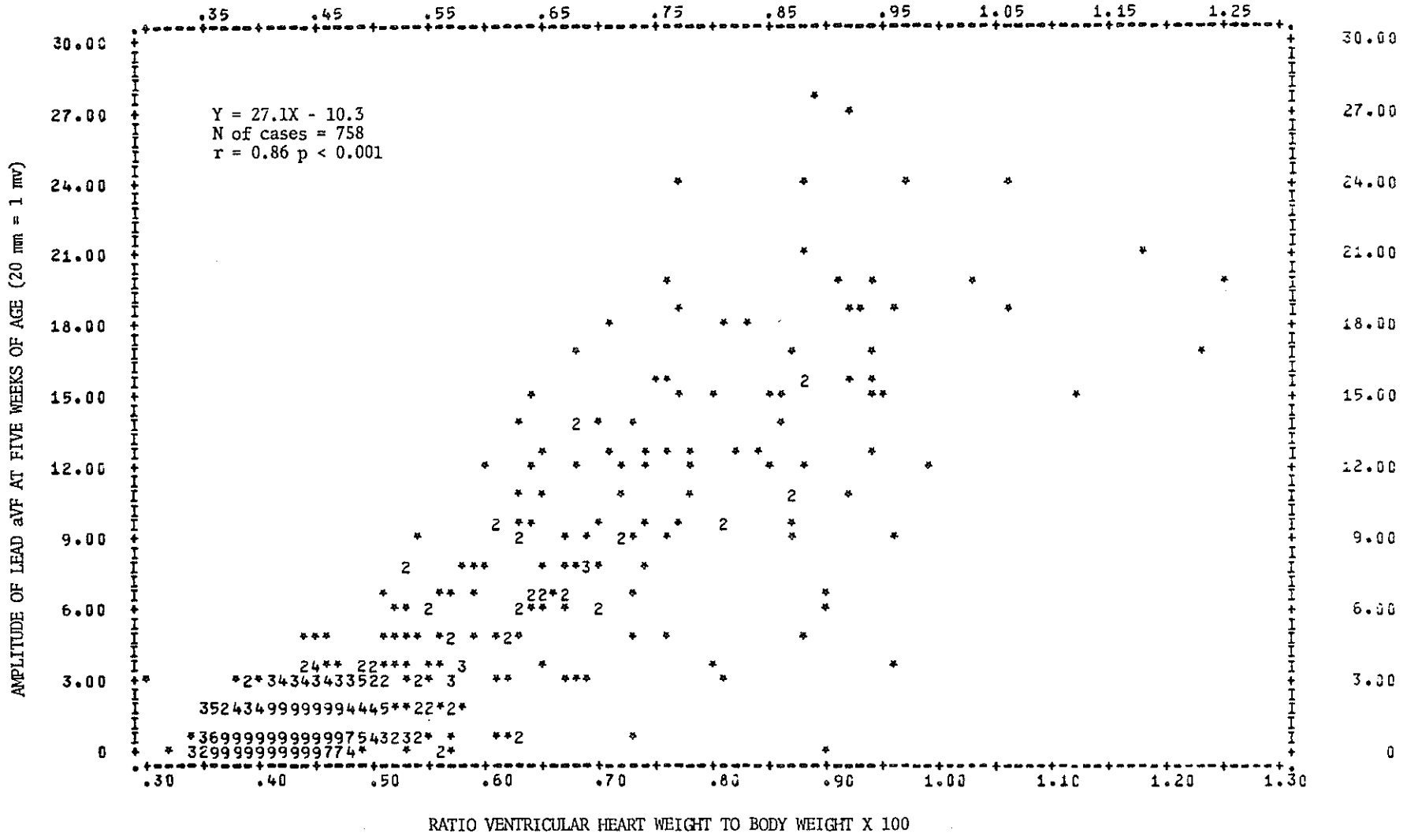


Figure 2: Distribution of lead aVF R wave amplitudes by ventricular to body weight ratios.



INTERRELATIONSHIPS BETWEEN PHOTOPERIOD AND AMBIENT TEMPERATURE ON LUTEINIZING HORMONE LEVELS IN BLOOD OF YOUNG TURKEYS

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The environment is an important factor regulating productivity in turkeys. The influence of light has been extensively investigated. Light does not only affect reproductive efficiency during the laying period, but its effect is carried over from the prebreeding period as well. The influence of ambient temperature on reproductive traits of laying birds is limited to the study of its effects during the laying period. It is well recognized that exposure of laying hens to high ambient temperature reduces egg production. Little is known about the effects of temperature during the growing and holding periods on subsequent reproductive performance.

Reproductive function is regulated by gonadotropic hormones. It follows that the measurement of their levels in birds exposed to differing temperatures may give us an insight into the expected effects of temperature on reproductive activity. Broad white male poults of the Nicholas strain were floor-reared in rooms with controlled light and temperature. Experimental photoperiods were imposed from the day of hatch onwards. At four weeks of age the temperature of the rooms was changed to the appropriate experimental conditions.

In experiment 1, turkey poults (4 weeks of age) were randomly allotted to four groups and maintained at 53°F, 60°F, 75°F or 86°F. Photoperiod was identical for all groups (16L:8D). Birds were castrated following 6 weeks exposure to experimental conditions. Blood samples were collected 6 and 14 days postcastration.

In experiment 2, day old poults were allotted to four groups under experimental photoperiods of 8 hrs light and 16 hrs of darkness daily (8L:16D), 12L:12D, 16L:8D and 20L:4D and the ambient temperature was maintained at 75°F. At 10 weeks of age, the birds were castrated and blood samples were taken 6, 10 and 14 days following castration.

In experiment 3, day old poults were reared under photoperiods of either 8L:16D or 20L:4D. At four weeks of age the birds in each group were divided into two subgroups and exposed to 60°F and 86°F respectively. This resulted in four experimental groups: 1) 8L:16D, 60°F; 2) 8L:16D, 86°F; 3) 20L:4D, 60°F; 4) 20L:4D, 86°F. When the birds were 10 weeks old, they were castrated and blood was taken at 0, 1, 3 and 7 days following castration for the determination of LH levels.

RESULTS AND DISCUSSION

The results of the first experiment (Fig. 1) demonstrate a progressive increase in serum luteinizing hormone in response to increasing temperature. Fourteen days after castration, the mean serum luteinizing hormone level of the 86°F group was significantly higher than that of the 53°F and 60°F

groups. Also, a significant difference existed between the 75°F and 53°F groups.

Serum LH levels of birds subjected to photoperiods of 8L:16D, 12L:12D, 16L:8D and 20L:4D are shown in Fig. 2. Increasing the hours of daily illumination caused serum LH levels to increase in a dose related manner at each of the three sampling times tested. Serum LH levels were greatest in birds maintained under 20 hrs of light and 4 hrs of darkness, daily. Turkeys exposed to a 8L:16D lighting regime showed the lowest LH levels of all groups. The levels of serum LH in the 12L:12D group were relatively lower than the levels in the 16L:8D group. These results clearly demonstrate that increasing photoperiods, given as a single continuous pulse of light between 8-20 hrs, further elevate serum LH levels, and support the concept that the mechanism that measures light duration does not operate on an all-or-none basis.

The degree of LH rise depends on the thermal environment. This effect appears to be dependent on the photoperiod. High ambient temperature further elevated LH levels under conditions of stimulatory photoperiod (20L:4D), whereas it had no effect under short photoperiod conditions (Fig. 3). Serum LH levels of turkeys subjected to 86°F and kept under 20 hrs of light were greater than in birds subjected to the same photoperiod and exposed to 60°F. Although, serum LH levels of the latter group were still greater when compared to that of birds maintained under a short photoperiod at both temperatures (Fig. 3). These findings indicate the lack of temperature effect under a short photoperiod. Further, they imply that high temperature can not substitute for long photoperiod, although, it has an augmentative effect on serum LH levels. Temperature, therefore, seems to play only a subsidiary role to photoperiod in LH release.

It is important that caution be applied in extending the interpretation of our findings to the influence of temperature on the reproductive efficiency in turkeys. Nevertheless, it may be of some value to consider the results of the present study in relation to the observed effects of season on sexual maturity following light stimulation. It is a common observation that breeder hens, light stimulated during the spring achieve sexual maturity sooner than hens light stimulated during the cold weather. One way in which sexual maturity is accelerated during the warm weather is by the rate of gonadotropin release.

It may be practical to subject breeder hens during the holding and early part of the breeding periods to relatively warm ambient temperature to achieve early sexual maturity. This may be followed by lower temperatures to extend the egg production season.

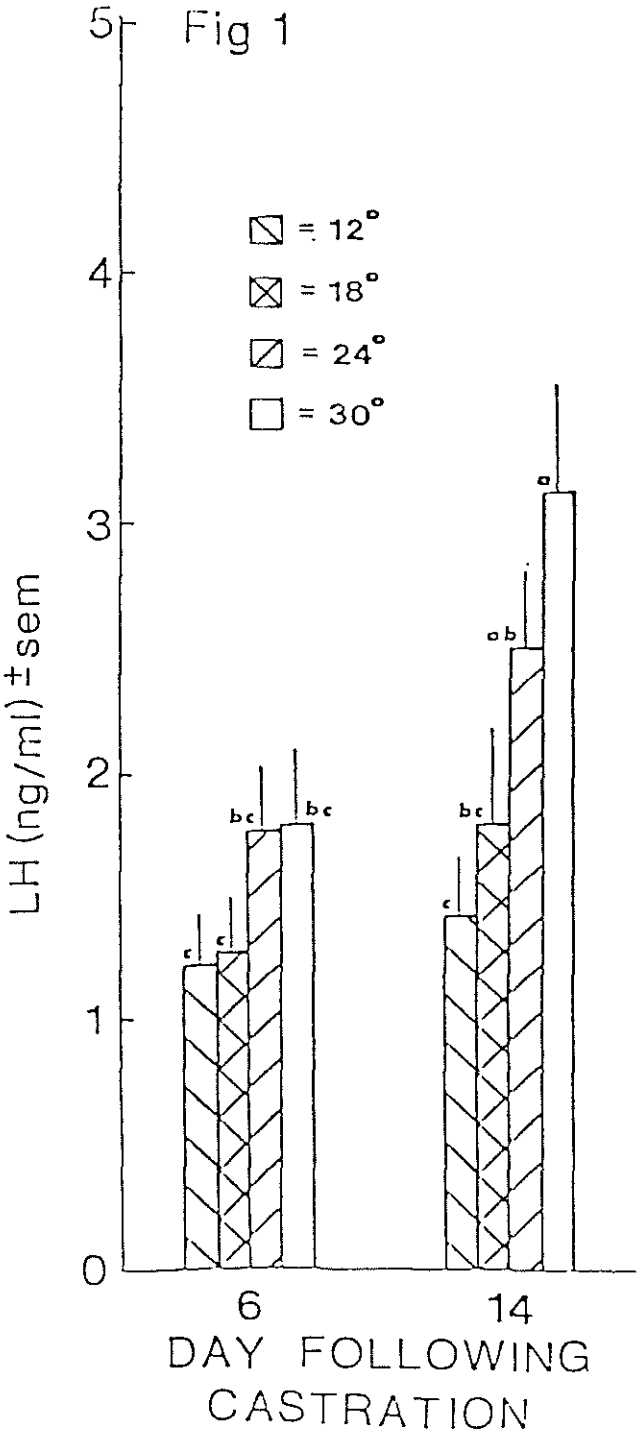

Fig. 1. Effect of ambient temperature on serum LH levels in juvenile gonadectomized male turkeys. Values given represent mean±S.E. of 7 birds/group.

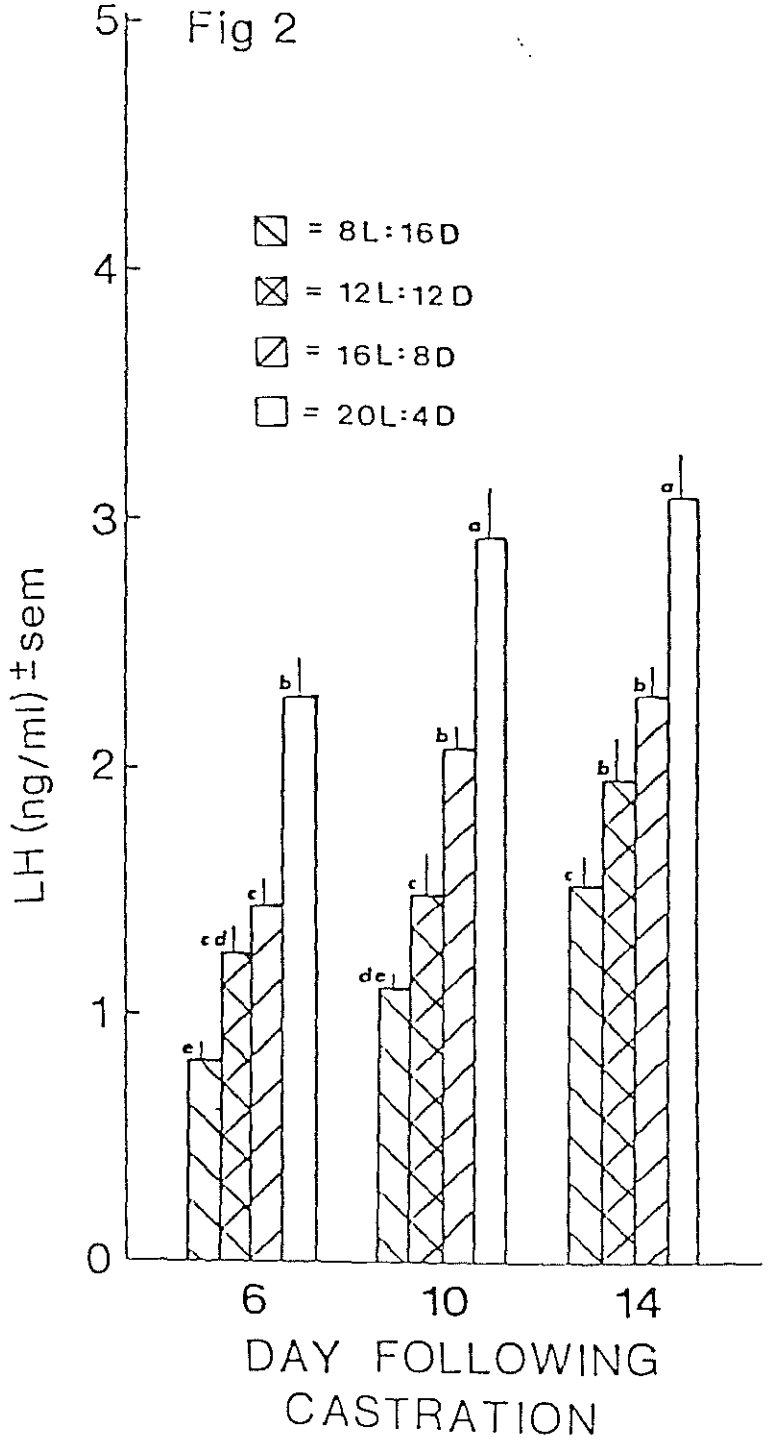

Fig. 2. Serum LH levels of juvenile gonadectomized male turkey, subjected to different lighting regimens. Number within columns indicate the gours of illumination. Values given represent mean±S.E. of 7 birds/group.

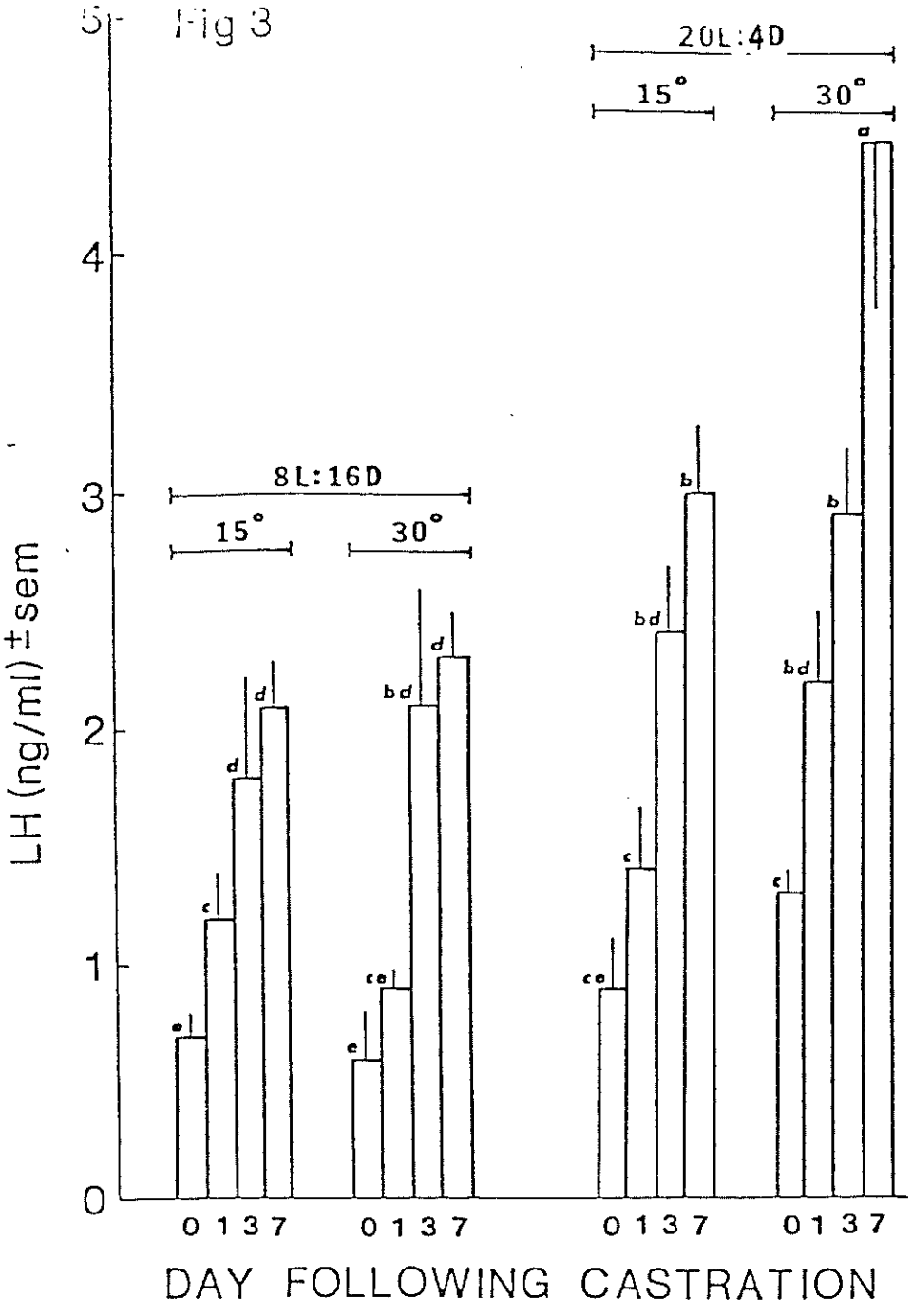

Fig. 3. Effects of various photoperiod-temperature regimens on serum LH levels of juvenile gonadectomized male turkeys. Values given represent mean±S.E. of 6 birds/group.