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REGULATION OF AVIAN GASTRIC FUNCTION: RECENT FINDINGS

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I and my colleagues have studied digestive function in turkeys and chickens for the last 20 years and we have previously summarized our findings in Agricultural Experiment Station reports (Duke, 1987; Duke and Dziuk, 1984) and elsewhere (Duke, 1982; 1983; 1986a; 1986b; 1987). The purpose of this paper is to update previous reports.

A major focus of our research has been to describe (eg. Duke et al., 1972; 1975; Dziuk and Duke, 1972) gastroduodenal contractile activity (i.e., motility) and then to determine how it is regulated (e.g., Duke and Evanson, 1972; 1976; Duke et al., 1976; 1979). We have also studied gastric secretion (Duke and Bedbury, 1985; Mosher and Duke, 1985). Both gastric motility and secretion are regulated by hormonal as well as neural means. The hormone avian pancreatic polypeptide (aPP) was found to depress both gastric motility and secretion in chickens (Duke et al., 1985). In humans and dogs pancreatic polypeptide (PP) does not affect gastric function. The upper portion of the gut of fowl is, however, quite different from that of mammals, in that it has a crop for storage of food and a gizzard for grinding food. So, differences in regulation between mammals and fowl are not too surprising. To pursue this apparent phylogenetic difference further we decided to determine the influence of aPP on gastric secretion and motility in Red-tailed hawks which, while they do have a crop, their gizzard is more comparable to the mammalian (non-ruminant) stomach. Also, hawks, humans and dogs eat discrete meals, whereas poultry tend to be continuous feeders. We found that aPP had no significant affect on gastric function in hawks (Duke et al., 1987a). Thus, the physiological role of PP appears to be more related to feeding habits and to gastric function than to taxonomic relationships.

As indicated above, PP has no effect on gastric function in mammals. The principal effect in mammals appears to be to suppress pancreatic and biliary secretion. To further examine relative differences in regulation of digestive processes between mammals and birds, we undertook to investigate the effect of aPP on these secretions in SCWL hens (Duke et al., 1987b). The hens were surgically prepared with cannulae for collecting secretions from the cystic duct and the duct draining the ventral pancreatic lobe, and for infusing the jugular vein with aPP or saline. An infusion rate which produced a plasma level of 15 ng of aPP/ml of plasma was used. A comparison of values obtained during saline infusion to those obtained during aPP infusion indicated that pancreatic and biliary secretory volumes and pancreatic total protein concentration were significantly depressed by aPP (Table 1), while the pH of pancreatic and biliary secretions was not significantly affected.
Thus, the role of aPP in regulation of pancreatic and biliary secretion appears to be similar in birds and mammals, however, unlike gizzard function, pancreatic and biliary functions also appear to be similar between mammals and birds.

In the studies described above, aPP was infused intravascularly (i.v.). It is also possible to administer drugs or hormones through an implanted cannula

into a cerebral ventricle (i.e., intracerebro-ventricular or ICV). In the former situation one tests "peripheral" responses to a hormone vs. "central" responses in the latter situation. Since aPP is found centrally in brain tissue and peripherally in pancreatic and intestinal tissue as well as in the blood, it is important to understand how it might regulate digestive processes centrally as well as peripherally. Dr. Mike Denbow from the Poultry Science Department at Virginia Polytechnic Institute, an expert in ICV administration of hormones, joined us for these studies and food intake as well as gastric function were studied. Centrally administered aPP was found to increase feeding but to have virtually no effect on gastric secretion and motility (Denbow et al., 1987). Thus, aPP does act centrally but its central and peripheral influences seem functionally inconsistent, i.e., why does this one hormone increase feeding when administered centrally, but decrease digestive function after peripheral administration? Obviously more study is needed.

Typically, gastrointestinal (GI) hormones are released post-prandially. Some are released by the act of eating, some by the presence of food in the stomach, some by the presence of food in the intestine, etc. These hormones both stimulate and inhibit digestive processes. The presence of food in the intestine releases aPP.

To gain some insight into the role of some of the other GI hormones in birds, we studied the effects of three common ones, cholecystokinin, secretin, and vasoactive intestinal peptide on pancreatic and biliary secretion (Duke et al., 1987c). Infusion (i.v.) of these hormones following a control infusion of saline indicated that they were primarily stimulatory (Table 2). Thus, since aPP was shown to be inhibitory, its apparent physiological role is to oppose or modulate the actions of other, stimulatory GI hormones. These three hormones did not affect the plasma concentration of aPP (Duke et al., 1986).

As indicated above, regulation may occur via neural or hormonal means. Regulation of GI motility may also be myogenic (i.e., arising from the muscle layers in the GI tract). In the mammalian GI tract an ongoing electrical activity can be recorded from the muscle layers which is called the "pacesetter potential". This arises from specialized cells called the "interstitial cells of cajal". We could not record this activity in the stomach of turkeys and, although we could record it from the duodenum, it did not appear to be "setting the pace" of duodenal contractions (Duke et al., 1972). We have hypothesized that the complex gastroduodenal contraction cycle of fowl is neurogenic, i.e., it arises from and is coordinated by the myenteric neurons (nerves lying between the longitudinal and circular muscle layers in the GI tract). We have recently begun studies to test this hypothesis.

In addition to the myenteric, or intrinsic nerves, the GI tract is also served by extrinsic nerves from the spinal cord and the base of the brain. These extrinsic nerves may both innervate GI muscles and connect with the myenteric nerves. As a first step in study of the influence of the myenteric nerves, we sought to determine the influence, if any, of the extrinsic nerves on initiation or coordination of the gastroduodenal contraction cycle. The vagus and two gastric branches of the sympathetic nerve were sectioned at the level of the glandular stomach. This denervation had no effect on the initiation of contractions, frequency of contractions (Table 3), nor on coordination of the gastroduodenal cycle (Chaplin and Duke, 1988). Thus, although the vagus and

sympathetic nerves are able to modulate gastroduodenal contraction frequency (Duke, 1986a), they do not appear to be essential for initiation or coordination of gastroduodenal contractions. This finding strengthens our hypothesis that myenteric nerves are responsible for this initiation and coordination.

It would have been difficult to test this hypothesis were it not for the recent work of Dr. Paul Bass and his colleagues in the Department of Pharmacology at the University of Wisconsin. This research team found that very dilute solutions of benzylkonium chloride (BC) selectively destroy nervous tissue without harm to other tissues (Dahl et al., 1987). This substance was applied to the surface of the rat intestine and nerves were destroyed without muscle being damaged.

Our first study with BC was aimed at determining whether the same results could be obtained in turkeys. First, BC was "painted" on the caudodorsal thin muscle of the gizzard of anesthetized, laporatomized turkeys. The turkeys were allowed to recover, then via radiographic observation of gastric motility we detected that the gizzard, proventriculus and duodenum were contracting normally except for the caudodorsal thin muscle which was not contracting at all (Chaplin et al., 1987). Subsequent gross post mortem examinations disclosed that, this muscle was flaccid and dilated. Microscopic exam indicated that nervous tissue had virtually disappeared from the muscle but the muscle tissue itself was normal. Thus, 1) BC does work on turkey neural tissues and 2) the finding that the thin muscle did not contract when its myenteric plexus was destroyed indicates that this plexus is necessary for contraction, i.e., initial evidence that our hypothesis is correct.

In a study currently underway, BC has been applied to the isthmus between the proventriculus and gizzard and to the pylorus to see if proventricular, gizzard and duodenal contractions become dissociated. Our results are not clear except that treatment of the isthmus causes a decrease in gastric frequency. Perhaps this region of the gastric area contains a neural "pacemaker" which was damaged by BC treatment. Further studies must be done.

In conclusion, I would like to quote from my last AES report in this subject:

> "There eventually will be significant advantages in knowing how digestive processes are regulated, and the relationship of hormones to this regulation seems especially important. Hormones that slow passage rate of food through the gut (aPP) or stimulate secretion of digestive enzymes could improve efficiency of utilization of diets. Hormones that stimulate appetite could be useful during anorexia. Recent research has discovered other hormones that influence intestinal absorption or secretion in mammals, these could be extremely useful during diarrhea. Feeding specific nutrients or combinations of nutrients that initiate release of hormones would avoid having to administer them. More information is needed, however, before we will be able to use hormones in this way. Thus, research such as described herein must continue."

LITERATURE CITED

Chaplin, S.B., and G.E. Duke, 1988. Effect of denervation on initiation and coordination of gastroduodenal motility in turkeys. Submitted to Am. J. Physiol. Mar., 1987.

Chaplin, S.B., G.E. Duke, H. Hunt, and L.A. Degernes, 1987. Chemical denervation of the myenteric plexus of the muscular stomach of turkeys. Accepted by Comp. Biochem. Physiol. Jan. 20, 1987.

Dahl, J.L., D.D. Bloom, M.L. Epstein, D.A. Fox and P. Bass, 1987. Effect of chemical ablation of myenteric neurons on neurotransmitter levels in the rat jejunum. Gastroenterol. 92:338-344.

Denbow, D.M., G.E. Duke, and S.B. Chaplin. Food intake, gastric secretion and gastric motility in chickens as affected by aPP administered centrally. Submitted to Physiol. Beh. May 10, 1987.

Duke, G.E., 1981. Regulation of Avian Digestion. Minn. Turkey Res., Minn. A.E.S. Report 179, pp. 13-17.

Duke, G.E., 1982. Gastrointestinal Motility and its Regulation. Gastrointestinal Symposium sponsored by Poult. Sci. Assn., Poult. Sci. 61:1245-1256.

Duke, G.E., Avian Digestion. Chapter 25, Duke's Physiology of Domestic Animals. 9th Ed. M.J. Swenson, Editor. pp. 313-320. Cornell U. Press (1977), and 10th Ed. (1983).

Duke, G.E., and H.E. Dziuk, 1984. Regulation of avian gastric digestion. Minn. Turkey Res., Minn. A.E.S. Report 30, pp. 71-75.

Duke, G.E., 1986a. Alimentary Canal: Anatomy, Regulation of Feeding, Motility. Chapter 11. Avian Physiology. 4th Ed., P.D. Sturkie, Editor. Springer-Verlag, New York, pp. 269-288.

Duke, G.E., 1986b. Alimentary Canal: Secretion and Digestion, Special Digestive Functions and Absorption. Chapter 12. Avian Physiology. 4th Ed., P.D. Sturkie, Editor. Springer-Verlag, New York, pp. 289-302.

Duke, G.E., 1987. Avian Gastrointestinal Motor Function. Chapter for, The Handbook of Gastrointestinal Physiology, Motility Section, J.T. Wood, Editor. Amer. Physiological Soc. Pub., accepted May, 1987.

Duke, G.E. and H.P. Bedbury, 1985. Lack of a cephalic phase of gastric secretion in chickens. Poult. Sci. 64:575-578.

Duke, G.E., S.B. Chaplin, H. Hunt, and L.K. Waird-Bauer, 1987a. The influence of avian pancreatic polypeptide on gastric secretion and motility in Red-tailed hawks (Buteo jamiacensis). Accepted by Comp. Biochem. Physiol. July 13, 1987.

Duke, G.E., H.E. Dziuk, and O.A. Evanson, 1972. Gastric pressure and smooth muscle electrical potential changes in turkeys. Am. J. Physiol. 222:167-173.

Duke, G.E., and O.A. Evanson, 1972. Inhibition of gastric motility by duodenal contents in turkeys. Poult. Sci. 51:1625-1636.

Duke, G.E., and O.A. Evanson, 1976. Diurnal cycles of gastric motility in normal and fasted turkeys. Poult. Sci. 55:1802-1807.

Duke, G.E., O.A. Evanson, and P.T. Redig, 1976. A cephalic influence on gastric motility upon seeing food in domestic turkeys, great-horned owls (Bubo virginianus) and red-tailed hawks (Buteo jamiacensis). Poult. Sci. 55:2155-2165.

Duke, G.E., J.R. Kimmel, H.P. Bedbury, and H.G. Pollock, 1985. The influence of avian pancreatic polypeptide on gastric secretion and motility in laying hens. Poult. Sci. 64:1231-1235.

Duke, G.E., J.R. Kimmel, H. Hunt, H.G. Pollock and J.A. Mosher, 1986. Influence of cholecystokinin, vasoactive intestinal peptide and secretin on plasma concentration of avian pancreatic polypeptide in laying hens. Comp. Biochem. Physiol., 85A:347-350.

Duke, G.E., J.R. Kimmel, K. Larntz, H. Hunt, and H.G. Pollock, 1987b. The influence of avian pancreatic polypeptide on pancreatic and biliary secretion in laying hens. Submitted to Poult. Sci. Mar, 1987.

Duke, G.E., J.R. Kimmel, P.T. Redig, and H.G. Pollock, 1979. Influence of exogenous avian pancreatic polypeptide on gastrointestinal motility of domestic turkeys. Poultry Sci. 58:159-166.

Duke, G.E., T.E. Kostuch, and O.A. Evanson, 1975. Gastroduodenal electrical activity in turkeys. Am. J. Dig. Dis., 20:1047-1058.

Duke, G.E., K. Larntz, and H. Hunt, 1987c. The influence of cholecystokinin, vasoactive intestinal peptide and secretin on pancreatic and biliary secretion in laying hens. Comp. Biochem. Physiol. 86C:97-102.

Dziuk, H.E. and G.E. Duke, 1972. Cineradiographic studies of gastric motility in the turkey. Am. J. Physiol. 222:159-166.

Mosher, J.A., and G.E. Duke. Cephalic control of avian gastric secretion. Comp. Biochem. and Physiol. 82A(4):935-937.

Table 1. Mean (± standard error) volume (µl/min), pH and total protein (TP) (mg/ml) concentration in the pancreatic secretion during infusion of saline followed by infusion of avian pancreatic polypeptide (aPP) (15 ng/ml of plasma) and mean (± standard deviation) difference in volumes, pH and TP between the saline infusion period vs. the last 20 min. of the aPP infusion period.

	Biliary					
	Saline		aPP		Diff.	
	$\bar{X}$	n[1]	$\bar{X}$	n	$\bar{X}$	n[2]
Vol.	4.9(±1.2)	10	2.6(±.9)	10	-2.3(±.9)*	10
pH	7.0(±.1)	7	6.9(±.1)	6	- .2(±.1)	6

	Pancreatic					
	Saline		aPP		Diff.	
	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	n
Vol.	6.0(±1.9)	6	1.9(±.7)	6	-4.1(±1.9)**	6
pH	7.6(±.1)	5	7.8(±.1)	4	.1(±.1)	4
TP	4.9(±.7)	5	3.2(±.8)	2	-2.1(±.1)*	2

[1]n=number of hens

[2]n=number of comparisons

*P<.05

**P<.01

Modified from Duke et al., 1987b.

Table 2. Estimated mean effects of infusion (i.v.) of cholecystokinin (CCK), vasoactive intestinal peptide (VIP) or secretin (Sec) following saline (Sal) infusion, on pancreatic and biliary volume and pH and on biliverdin and total protein concentration.

	Biliary			Pancreatic		
	Volume	pH	Biliverdin	Volume	pH	Tot. protein
Sal effect	+0.053	+0.120	---	+0.074	+0.057	-0.40
SE (eff.)†	(0.346)	(0.164)	---	(0.225)	(0.119)	(4.13)
Exp. eff.†	+5.4%		---	+7.7%		
t	+0.15	+0.73	---	+0.33	+0.48	-0.10
CCK effect	+0.346	+0.034	+0.134	-0.034	+0.086	+1.96
SE (eff.)	(0.150)	(0.076)	(0.076)	(0.096)	(0.060)	(4.11)
Exp. eff.	+41.3%*		+8.9%*	-3.3%		
t	+2.30*	+0.46	+1.76	-0.35	+1.44	+0.48
VIP effect	+0.254	+0.177	-0.214	+0.303	+0.106	+2.61
SE (eff.)	(0.164)	(0.078)	(0.076)	(0.108)	(0.057)	(3.56)
Exp. eff.	+28.9%		-19.3%*	+35.4%*		
t	+1.55	+2.27*	-2.83*	+2.81*	+1.88*	+0.73
Sec effect	-1.058	-0.020	-0.845	-0.036	+0.214	-1.70
SE (eff.)	(0.301)	(0.164)	(0.553)	(0.199)	(0.084)	(7.12)
Exp. eff.	-65.3%*		-57.1%	-3.5%		
t	-3.51*	-0.12	-1.53	-0.18	+2.54*	-2.39

*P<0.05

†Standard error of effect

†Exponential effect (antilog of logarithmic effect) expressed as percentage. Pancreatic total protein concentration and pH effects are also percentages.

Modified from Duke et al., 1987c.

Table 3. Comparison of mean frequency of gastric contractions per minute (motility) in four denervated and four sham-operated turkeys.

	DENERVATED		SHAM	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Fed Motility				
Day 1	1.8	(0.6)	1.9	(0.5)
Day 3	3.4	(0.7)	3.7	(0.5)
Day 5	3.1	(0.6)	3.6	(0.6)
Day 7	3.0	(1.2)	3.1	(0.6)
Day 14	3.3	(0.4)	3.4	(0.5)
Fasted Motility	1.0	(0.3)	1.2	(0.6)

Modified from Chaplin and Duke, 1988.

MODELING TURKEY GROWER BARN VENTILATION

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ABSTRACT

Ventilation and its impact on bird health, production energy costs and litter conditions are important to turkey producers. A model was developed to estimate inside temperature, relative humidity, litter moisture content, ventilation rate and supplementary heat. It includes a finite element method subroutine to describe moisture movement into and out of the litter. Initial results indicate that litter moisture diffusivity has a significant effect on the litter moisture content and a minimal effect on the ventilation rate and supplementary heat. Bird age and ambient temperature had a minimal effect on the litter moisture content and a significant effect on the ventilation rate and supplementary heat. Basic research to determine diffusivity and other physical parameters is needed.

INTRODUCTION

The economics of competively raising turkeys in Minnesota requires extensive use of confinement facilities for year round production. Increased use of confinement has been accompanied by significant increases in the occurrence of air sacculitis problems. These problems have been caused by ubiquitously distributed organisms such as E. coli, which causes severe septicemic desease, and Aspergillus fumigatus, which causes a chronic disease in male turkeys and frequently causes mortalities and condemnations of 5-10% in production flocks of male birds [1]. Neither female turkeys reared under similar circumstances, nor male turkeys reared on range, are affected to the same extent [2].

In 1984 an interdisciplinary team of researchers began to study the complex problem of air sacculitis caused by Aspergillus fumigatus. It was proposed that a complex interaction of intrinsic sex-related susceptibility and unfavorable environmental conditions combined to render the male turkey susceptible to this ubiquitously distributed organism of otherwise low pathogenicity. One part of the research effort was to develop a computer model of ventilation of turkey grower facilities. The ventilation model was to describe the environmental conditions and air quality in confinement turkey barns. Ultimately the model was to be used to relate ventilation and air quality to turkey health and production.

The purpose of this paper is to describe the development of a time dependent computer model of wintertime ventilation of turkey grower facilities. The model was to handle typical conditions and management found in Minnesota and include the movement of moisture into and out of the litter. The model was also to permit a variety of management systems and ambient weather conditions to be used so that evironmental conditions and supplementary energy use could be evaluated.

LITERATURE REVIEW

Information regarding typical wintertime ventilation management of turkey grower barns in Minnesota was obtained through informal discussions with producers, farm managers, animal scientists, veterinarians and agricultural engineers. Through these discussions about turkey ventilation management, the importance of litter moisture conditions on ventilation management became apparent. If the litter was considered to be to wet by the manager, the ventilation rate was increased to dry it. If the litter was to dry, the ventilation rate was decreased to save energy. This interrelationship between litter moisture content and ventilation rate was considered to be a key element that any model developed had to include to be of any substantial value.

Numerous models have been developed relating ambient environmental conditions to the conditions in a confinement building [3-8]. Most use steady state sensible heat balances to relate indoor temperature and supplementary heat needs to the external temperature, building design, insulation, ventilation rate and animal size and number housed in the building [6-8]. Others used time dependent sensible heat equations to develop dynamic models [3-5]. Some models also incorporate animal production interactions as a function of internal environmental conditions [4,5]. Only a few models include moisture balances in their analysis even though such equations are well established [4,7]. Moisture is an important factor in ammonia generation and leg problems in poultry facilities that use litter [9,10]. Dynamic moisture movement through litter has not been modeled previously.

MODEL DEVELOPMENT

The dynamic model was developed by writing sensible heat and moisture balances for a representative turkey grower barn. The moisture balance incorporated a subroutine using the finite element method to describe the moisture movement into and out of the litter. To include ambient weather data, hourly wintertime temperature and relative humidity data was inputted. Turkey growth was accounted for by weekly adjusting bird age; bird weight, sensible heat, moisture production, and moisture from spilled water and voided feces. The model accomodated a variety of ventilation management systems and limits. Model outputs were; inside temperature, inside relative humidity, litter moisture content, supplementary heat and ventilation rate. Variables are defined in the table of symbols.

Sensible heat balance

The time dependent sensible heat balance equation used was:

$$\frac{V}{\upsilon}\frac{\partial h}{\partial t} = q_v + q_s + q_{sup} - q_b \qquad (1)$$

The left side of the equation accounts for the change in sensible heat in the air within the building. Sensible heat changes due to temperature changes in equipment and building components was neglected. The partial derivative of the enthalpy was approximated using the finite difference method:

$$\frac{\partial h}{\partial t} = \frac{h(t + \Delta t) - h(t)}{\Delta t} \qquad (2)$$

The sensible heat used to heat the ventilation air was calculated using:

$$q_v = \frac{Q}{\upsilon}(h_i - h_{tp}) \qquad (3)$$

Weekly bird weights were based on recent growth data [11]. Values for 14 and 18 week old toms are listed in Table 1. Total heat was assumed to be 80 percent sensible heat and 20 percent latent heat (moisture) and generated at a rate of 5.8 W/kg of turkey for birds over 13 weeks of age.

The supplementary heat calculated by the model was the amount needed to maintain the inside temperature at the minimum.

Building heat loss was calculated using;

$$q_b = UA\,(T_i - T_o) \qquad (4)$$

Moisture balance

The time dependent moisture balance expression used was:

$$\rho\, V_l \frac{\partial C}{\partial t} + \frac{V}{\upsilon}\frac{\partial w}{\partial t} = M_g - M_v \qquad (5)$$

The first term on the left side of equation (5) was the change in litter moisture content. It was calculated using the procedure described in the next section. The partial differential term describing the change in moisture in the air was approximated using the finite difference method:

$$\frac{\partial w}{\partial t} = \frac{w(t + \Delta t) - w(t)}{\Delta t} \qquad (6)$$

Table 1 lists the moisture production per bird at 14 and 18 weeks of age. The combined total water spilled and voided in the feces that needed to be evaporated is also listed in Table 1.

The moisture removed by the ventilation air was calculated using:

$$M_v = \frac{Q}{v} (w_o - w_i) \qquad (7)$$

Litter moisture content

Litter moisture content was calculated in a study to determine factors affecting ammonia production in broiler houses using equation (8). The relative humidity had been assumed to be 70 percent and the ventilation rates were preset each week.

$$M_E = \left[\frac{-\ln (1 - rh)}{cT} \right]^{1/n} \qquad (8)$$

where: rh = relative humidity (decimal)
T = absolute temperature (R)
c and n = constants for wood, 5.34×10^{-6} and 1.41, respectively.

The method used in this study to model the time dependent moisture movement in turkey litter was based on Fick's second law of diffusion in one dimension:

$$\frac{\partial C}{\partial t} = D_{ab} \frac{\partial^2 C}{\partial x^2} \qquad (9)$$

A subroutine using the finite element method was used to solve this equation. The transient concentrations were calculated using the central difference method [12]. To insure continuity between the surface litter moisture content and the inside air relative humidity, equation (8) was used to set the litter moisture content at the surface in the finite element subroutine. Spilled and feces water that was to be evaporated was considered to be a source at the second node, 0.5 cm below the surface of the litter. The surface node could not used because it would have interferred with the boundary condition set by equation (8).

Litter depth was set at 15 cm. Twenty one nodes were used, with the top ten spaced at 0.5 cm increments while the bottom ten were spaced at 1.0 cm increments.

Equation (9) required a diffusion coefficient for moisture movement through turkey litter. Drying times for chicken manure had been studied but wood chips had not been included with the manure and diffusivity had not been determined [13]. To obtain representative values an undergraduate research project was conducted. Diffusivities for turkey litter ranged from 4×10^{-6} to 3×10^{-5} cm^2/s [14].

Ambient temperature and relative humidity data

Hourly temperature and relative humidity data for representative winter months were inputted and stored in a data file. The file contained both below normal and normal temperature data. The below normal temperature data used ranged between -27 and -5 C with a ten day average of -17 C. The normal temperature data ranged from -24 to 1 C with an average of -10 C.

Solution procedure

Equations (1) - (9) provide a system of three independent equations with five possible unknowns; inside temperature, inside relative humidity, ventilation rate, supplementary heat and litter moisture content. To obtain solutions the ventilation system limits in Table 1 and typical ventilation control strategies were used.

An iterative solution procedure was used each time step to solve the system of equations. Solutions found were compared to the ventilation system limits. A solution was accepted if it fell within the ventilation system limits and the management strategy guidelines. Once the results were accepted, the next time step was begun by updating the temperature and production inputs.

RESULTS AND DISCUSSION

The purpose of this model was to describe ventilation of turkey grower barns including the moisture movement into and out of the litter. The factors considered for this report were the litter moisture diffusion coefficient, bird age and outside temperature. The end points considered for comparions were; the litter moisture content 1.0 cm below the surface, inside temperature and relative humidity, supplementary heat needed and the average ventilation rate.

Litter moisture diffusion

Preliminary results obtained during development of the litter moisture content subroutine had indicated that the diffusivity value had an significant impact on the nominal litter moisture content. High diffusivities (easier moisture transfer) produced lower nominal litter moisture contents while low diffusivities resulted in higher litter moisture contents. Figure 1 shows the litter moisture content at 1.0 cm below the surface versus time using a diffusivity of 1.7×10^{-5} cm^2/s and below normal ambient temperature data. The litter moisture content ranged from 15 to 16.3 percent wet basis. Using a diffusivity of 3.0×10^{-5} cm^2/s produced litter moisture contents between 10 and 12.5 percent wet basis. Results obtained using a diffusivity of 4.0×10^{-6} cm^2/s resulted in litter moisture contents around 36 percent wet basis.

Figure 1 also shows the inside relative humidity versus time. The litter moisture content paralleled the inside relative humidity very closely after conditions had stabilized from initial values.

The litter moisture content maximums and minimums lagged behind the relative humidity maximums and minimums by about 2-4 hours.

The average ventilation rate, inside temperature and supplementary heat were similar for all three diffusivity values. The below normal outside temperature data kept the ventilation rate and inside temperature at the minimum values allowed. The supplementary heat summed to around 45 GJ over the ten day period analyzed.

Bird age

Bird age affected the sensible heat, moisture production and allowable inside temperatures used as inputs to the model. Figure 1 showed the results using 14 week old toms. Figure 2 shows the litter moisture content and inside relative humidity results obtained for 18 week old toms using the below normal ambient temperature data. Figure 2 shows the paralleling between the litter moisture content and inside relative humidity seen previously. After an initial continious increase in litter moisture content, it fluctuates between 16.5 and 17.5 percent wet basis. The slightly higher litter moisture content obtained with the older birds was confounded with the change in allowable inside temperatures for the older birds. This is a common practice in Minnesota in the winter because the lower inside temperature saves energy and increases the inside relative humidity which reduces the dust level in the barn.

As expected, the ventilation rate was higher and the supplementary heat needed was lower for the older birds. The 14 week old birds used the minimum ventilation rate, 2.7 m^3/s, continuously while the rate ranged from 2.7 to 5.5 m^3/s and averaged 3.6 m^3/s for the 18 week old birds. The 14 week old birds required 45.7 GJ of supplemental heat while the 18 week old birds used 0.85 GJ. Again this effect was confounded with the change in allowable inside temperature.

Ambient temperature

Results using the normal January temperature data produced expected increases in the inside temperature and ventilation rate and a decrease in the supplementary heat for both the 14 and 18 week old birds. The normal temperature 14 week old birds had an inside temperature of 21 C whereas the cold weather data essentially did not deviate from the minimum inside temperature of 18.5 C. The ventilation rate during the normal temperature averaged 3.0 m^3/s, reaching as high as 4.4 m^3/s, whereas the cold temperature data required the minimum ventilation rate of 2.8 m^3/s continuously. Energy use during the normal temperature data was 18.6 GJ, almost 60% less than the 45.7 GJ used during the cold temperature analysis.

The 18 week old birds also required more ventilation, averaging 4.9 m^3/s during the normal January compared to the 3.6 m^3/s averaged during the below normal analysis. No supplemental energy was needed using the normal temperature data.

The ambient temperature affects on the litter moisture content were different for the two bird ages. The 14 week old birds had a

litter moisture content increase of 0.3 percent wet basis between the cold and normal January temperature data. The 18 week old birds had a decrease of 0.6 percent wet basis. These results occur in part because of the effects that inside temperature and relative humidity had on the equilibrium moisture content at the litter surface. These results indicate complex interactions exist between weather, bird age, ventilation and the litter moisture content.

Initial litter moisture content

The selection of the initial litter moisture content had a significant impact on the results over a limited number of days. Selecting an initial moisture content far from the nominal value resulted in a steady increase or decrease in the moisture content until it reached the nominal value. This can be seen in Figure 2 during the first 40 hours of analysis. The duration of the increase or decrease depends on the diffusivity and deviation from the nominal value.

The initial moisture content values used for these results were below and within 2% of the average litter moisture content values so that it stabilized within 48 hours.

CONCLUSION

A turkey ventilation model was developed using sensible heat and moisture balances to estimate inside temperature, relative humidity, litter moisture content, ventilation rate and supplementary heat. The moisture balance includes a finite element method subroutine to describe moisture movement into and out of the litter. The model uses stored hourly ambient temperature and relative humidity data and weekly adjusted turkey production data as inputs. It accomodates various ventilation systems and management schemes. Results from the model indicated that the litter moisture diffusivity was a very important parameter affecting the nominal litter moisture content. The litter moisture content paralleled the inside relative humidity. Factors that affect the inside relative humidity in turn will affect the litter moisture content. Bird age and ambient temperature had a greater impact on the ventilation rate and supplemental heat needed than on the litter moisture content. Basic research to determine the appropriate diffusivities and equilibrium relationship is needed.

TABLE OF SYMBOLS

C = litter moisture content (percent wet basis)

D_{ab} = moisture diffusion coefficient for turkey litter (cm^2/s)

h_i = inside air enthalpy (kJ/kg DA)

h_o = outside air enthalpy (kJ/kg DA)

h_{tp} = turning point enthalpy of the air, evalulated at the outside humidity ratio and the inside temperature (kJ/kg DA)

M_e = equilibruim moisture content (percent dry basis)

M_g = moisture generation (kg/s)
M_v = moisture removed by ventilation air (kg/s)
q_b = heat loss through walls, roof, doors, etc. (kJ/s)
q_s = sensible heat produced by the animals (kJ/s)
q_{sup} = supplementary heat (kJ/s)
q_v = sensible heat used to heat the ventilation air (kJ/s)
Q = ventilation rate (m^3/s)
t = time (s)
$t+\Delta t$ = time at end of the current time step (s)
T_i = inside air temperature (C)
T_o = outside air temperature (C)
UA = heat loss factor determined from the building situation (kW/C)
υ = inside air specific volume (m^3/kg DA)
V = building air volume (m^3)
V_l = litter volume (m^3)
w_i = inside air humidity ratio (kg/kg DA)
w_o = outside air humidity ratio (kg/kg DA)
x = distance (cm)
ρ = litter density (kg/m^3)

REFERENCES

1. Richard, J.L., R.C. Cutlip, J.R. Thurston, and J. Songer. 1981. Response of turkey poults to aerosolized spores of Aspergillus fumigatus and aflatoxigenic and nonaflatoxigenic strains of Asperigillus flavus. Av. dis. 25(1):53-67.

2. Nivas, S. 1985. Aspergillosis in winter market toms in Minnesota: How to prevent it. Gobbles 39(5):6-8.

3. Albright, L.D. and N.R. Scott. 1974. An analysis of steady periodic building temperature variations in warm weather - Part I: A mathematical model. Trans. of the ASAE 17(1):88-92,98.

4. Stevens, G.R., J.A. DeShazer, T.L. Thompson and N.C. Teter. 1976. Environmental control for swine housing based on energy conservation and animal performance. ASAE Paper No. 76-4028. ASAE, St. Joseph, MI 49085.

5. Nass, I.A. and M.L. Esmay. 1981. Computer simulation of swine housing temperatures and heat production under tropical conditions. ASAE Paper No. 81-4562. ASAE, St. Joseph, MI 49085.

6. Timmons, M.B. and R.W. Bottcher. 1981. Finite element thermal analysis of livestock housing. ASAE Paper No. 81-4025. ASAE, St. Joseph, MI 49085.

7. Reese, F.N. and B.D. Lott. 1982. Mathematical model of broiler chicken houses. In: Livestock Env. II, Proc. 2nd Int. Livestock Env. Sym., ASAE, St. Joseph MI. pp 241-246.

8. Jones, D.D., W.H. Friday, C.N. Hinkle and L. Milman. 1982. Small computers for evaluatint energy use in livestock buildings. In: Livestock Env. II, Proc. 2nd Int. Livestock Env. Sym., ASAE, St. Joseph MI. pp 268-277.

9. Elliott, H.A. and N.E. Collins. 1982. Factors affecting ammonia release in broiler houses. TRANS. of the ASAE 25(2):416-417.

10. Martland, M.F. 1985. Ulcerative dermatitis in broiler chickens: the effects of wet litter. Avian Path. 14:353-364.

11. Sell, J.L. 1985. Notable improvements in 1984 turkey weights. Turkey World, Jan-Feb.

12. Segerlind, L.J. 1976. Applied Finite Element Analysis. John Wiley and Sons, Inc.

13. Sobel, A.T. 1969. Removal of water from animal manures. Cornell Waste Management Conference on Animal Waste Management. Cornell University, Ithaca, New York.

14. Moore, J.D. 1986. Moisture diffusion through turkey litter. UROP Report, Agricultural Engineering, University of Minnesota, St. Paul, MN.

Table 1. Model inputs and limits.

	Bird age (weeks)	
	14	18
Bird weight (kg	7.2	10.4
Sensible heat production (W/bird)	33.3	48.6
Moisture production (g/hr-bird)	12.3	17.9
Spilled water and feces moisture (g/s)	19.0	19.0
Inside temperature range (C)	18.5-21	15-18
Number of birds	5000	5000
Minimum ventilation rate (m^3/s)	2.8	2.8
UA (W/C)	1125	1125
Maximum ventilation rate (m^3/s)	47.2	47.2
Maximum supplementary heat (kW)	117	117

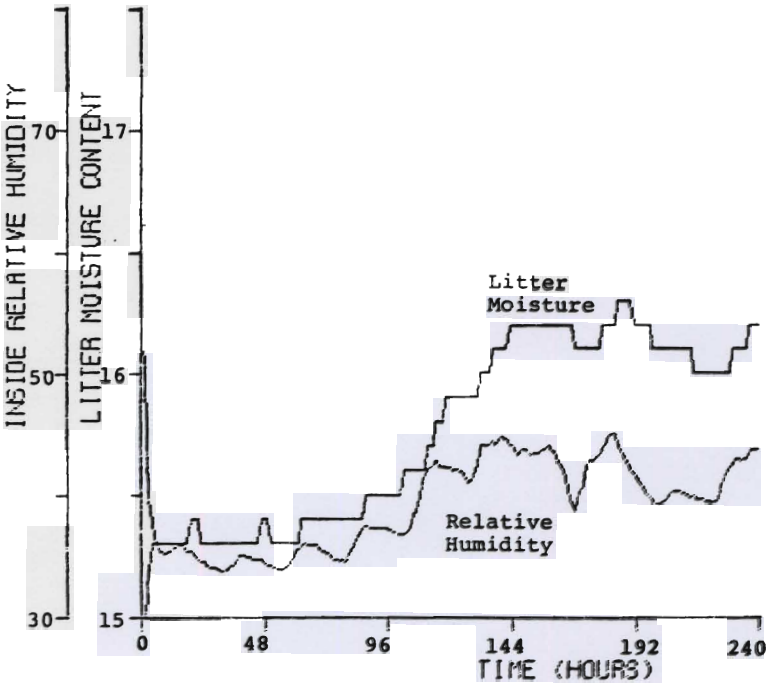

Figure 1. Model results for 14 week old turkeys using a diffusivity of 1.7 x 10^{-5} cm^2/s and below normal January temperatures.

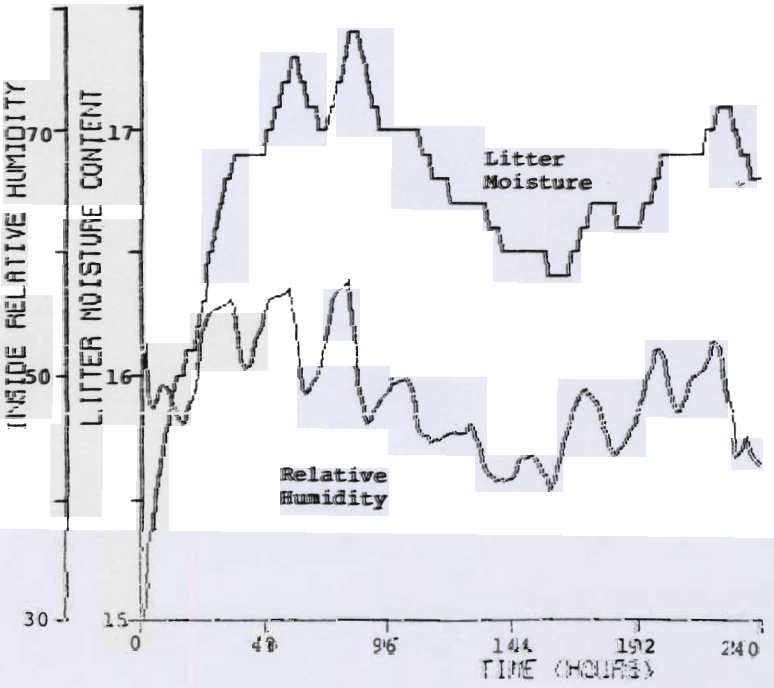

Figure 2. Model results for 18 week old turkeys using a diffusivity of 1.7 x 10^{-5} cm^2/s and below normal January temperatures.

NUTRITION, ENVIRONMENT, AND CARCASS QUALITY IN TURKEYS

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There are varying aspects to carcass quality, including proportions of fat and protein, weights of body components, fatty acids and other nutrient composition, stability, flavor, and texture. These add up to nutritional value and consumer acceptability.

What are the controlling factors for these characteristics? Surely the greatest immediate and alterable factor is nutrition. The most important long range factor is genetics. Physical environment and bird health are influential.

Not too long ago carcass quality was readily associated with the "lack of finish" problem in roaster-fryer turkeys marketed at an early age and about 10 pounds live weight. Now, with the whole large bird, turkeys have too much abdominal fat. These are important considerations when marketing the whole bird.

With further processing, primary interest is in turkey parts. Strains with more white meat are valued. The types of fatty acids laid down and carcass stability will be ever more important.

There is also the need to produce turkey meat with the greatest efficiency of calorie and other nutrient use. It is very inefficient to produce birds with excessive carcass fat. So even though only the breast, thigh, or wing may be used, a bird with unwanted fat is looked upon with disfavor.

The Minnesota turkey nutrition and management research program has over the years pursued many experimental objectives with limited personnel. There has not been the physical time or cooperative relationships to do further carcass studies. The bird is weighed at market time and sent to the processing plant with all its valuable information hid under its feathers.

There are three areas, however, where we have been able recently to do further carcass studies, and these will be summarized herein. They are: (1) influence of compensatory growth on carcass changes; (2) influence of types of diets and environments on carcass composition; and (3) influence of types of fat and levels of supplemental vitamin E on carcass stability and vitamin E liver storage.

I. INFLUENCE OF COMPENSATORY GROWTH ON CARCASS COMPOSITION

The object of the study was to determine growth and body composition recovery and influence on feed efficiency in Nicholas turkeys following protein restriction during 0-6 weeks of age. The restriction selected was to reduce protein so that the lysine level was 66% of control (per diet metabolizable energy). Beginning at 43 days of age all turkeys were fed the same diets. With toms, diets were modified at 4 week intervals; with hens, after 8 weeks of age, diets were modified at 3 week intervals. Animal fat level was 2% until 8 weeks of age, 4% in the 3rd and 4th

periods, and 6% in the 5th and 6th periods, respectively. Tom and hen turkeys were held to 24 and 20 weeks of age, and selected data are depicted graphically in figures 1 and 2, respectively.

The results were similar for toms and hens, so will be described together. Growth curves are shown in the "a" graph. Following marked growth depression with the low protein diets, turkeys did not begin catching up until considerably after 6 weeks of age. However, compensation did occur, so that by the end of the studies the restricted groups caught up with the controls.

Shown in the "b" graphs are breast weights expressed as a percentage of body weight. As with body weights, breast weights caught up (statistically) by the end of the study.

Shown in the "c" graphs are the body fat weights on a percentage basis. During restriction the low protein birds were fatter, in keeping with expected calorie/protein effects on carcass fat. However, when returned to the control diet these differences disappeared quickly. There was no effect of early restriction on future fat deposition. The dramatic increase after 8 weeks of age was similar in both treatments, suggesting that the number of adipocytes was unaffected by the level of protein restriction herein.

Shown in the "d" graphs is carcass protein. The early differences (lower carcass protein with restricted diet protein) disappeared with time.

The feed efficiency differences in this study were interesting. For control and restricted treatments, males at 24 weeks of age had feed/gains of 3.12 and 2.95, and females at 20 weeks of age had feed/gains of 3.08 and 2.97, each respectively.

II. INFLUENCE OF DIET AND ENVIRONMENT ON CARCASS COMPOSITION

A comprehensive study has been conducted on the influence of environment and diet on body weight and carcass characteristics of Nicholas tom turkeys on treatment from 4 to 20 weeks of age. Other details of this study were presented by Noll et al. (1987).

The environments were programmed into the 4 rooms of the Environment Building at the Rosemount Turkey Research Unit. Three rooms received intermittent (I/, as abbreviated in Figure 3) lighting (2L:4D 4X daily). One room was kept at 7 C, one at 21 C, and the third room cycled (/C) in temperature (2 hours at 7 C; 4 hours at 21 C 4X daily) in conjuction with the intermittent schedule indicated. The fourth room had cycling (/C) temperatures, as the 3rd room, but lighting was continuous (C/) (18L:6D daily).

There were 16 pens in each room. Four diets were fed, with 4 pens per diet. In each 4 pen group, two pens were stocked at high density (.21 sq. m./bird) and two pens at low density (.45 sq. m./bird). The diets included a control corn-soybean meal series with 1% added fat (diets were modified at 4 week intervals). A second treatment was the identical diet, but in pelleted form. A third treatment included 2, 4, 6, and 8% added fat during the 2nd through 5th periods. The fourth treatment included 20, 35, 50, and 65% barley fed during the 2nd through 5th periods. Fat and barley were added so as to keep critical nutrient:energy ratios constant.

Main effect body weights are presented in Figure 3A. Gains were better and poorer at 7 and 21 C, respectively, compared to cycling temperatures. Regarding the types of diets, pellets and fat resulted in superior gains, while birds fed barley were similar to those fed corn. Birds at low population density were heavier than birds at high density. There were no significant interactions among environment, diet, and density.

Abdominal fat pad data are presented in the same manner as body weight data in Figure 3B. The association of body weight with fat pad weight is striking. Exceptions to this relationship are (1) that pellets did not affect fat pad weight very much although they had resulted in a sizable body weight gain response; and (2), that diet barley resulted in significantly depressed percentage abdominal fat pad weight.

The protein and fat compositions as a percentage of dry matter for the four environments (main effects) are shown in Fig. 4A. Turkeys in the warm environment had greater carcass protein and lesser carcass fat levels than turkeys in the cool environment.

Figure 4B shows that barley supported the deposition of greater protein and lower fat levels in carcasses. These effects were followed in order by the corn-soy control, corn-soy pelleted, and high fat diets. When fat was higher, protein was lower, as expected on a dry matter basis.

There were no significant ($p < .05$) interactions among environment, diet, and density in any of the above-mentioned measurements.

Carcass moisture (%) also showed main effect differences with no significant interactions. Where carcass % fat levels in figures 4A and 4B were higher, there was generally reduced carcass moisture. This is expected as fat is a major component which displaces other components, the greatest of which is carcass moisture.

III. INFLUENCE OF DIET FAT TYPE AND VITAMIN LEVELS ON CARCASS STABILITY AND HEPATIC VITAMIN E

With more turkeys being marketed as fresh product, in processed or pre-cooked forms, the stability of carcass meat has taken on renewed importance. Turkey is known to have relatively poor natural stability (Mecchi et al., 1956). Supplemental vitamin E delayed the onset of rancidity and protected against "warmed over" rancidity in cooked meats as measured by the thiobarbituric acid test (Marusich et al., 1975).

Rethwill et al. (1983) reported that high vitamin E exerted a great effect in retarding oxidative rancidy in carcasses and that the effect was modifiable by dietary fat. Four diet series were fed: 1) control, corn-soy, no added fat (C); 2) corn-soy with 8% hydrolyzed animal-vegetable fat (AV); 3) corn -soy with 8% soybean oil (SO); and 4) corn-soy with 8% animal fat (A).

The TBA data in the Rethwill study are shown in Table 1. The TBA analyses were for (non-frozen) turkeys stored in a refrigerator for 3 weeks. The 10 IU/kg supplemental level of vitamin E is of particular interest as it is close to usual vitamin E supplementation in practice; here, TBA values were higher with AV and A and lower with C and SO. Meat having 3.0 and 2.6 TBA values with AV and A, respectively, was considered

to be quite oxidized. The 10 IU vitamin E/kg level did not protect against rancidity development nearly as well as did the 300 IU/kg level.

The positive relationship between diet vitamin E and hepatic storage of vitamin E is apparent. Of particular interest again is the effect with the approximate usual level of 10 IU vitamin E per kg diet. Hepatic E was relative high with the corn-soy diet series. It was intermediate with SO or A; however, it was low with AV.

Analytical data provided in Rethwill et al. (1983) showed that the AV fat used contained 55% free fatty acids and only 12% linoleic acid, while the animal fat probably contained poultry fat as indicated by its linoleic acid content of 10%. These analytical values are important as the AV and A fats used in the present work were not judged to be typical of what is usually fed in practice. They were, however, obtained from local turkey farmers as described.

The correlation between muscle TBA values and hepatic vitamin E levels was -.942, and was highly significant. The depressing effect of hydrolyzed animal-vegetable fat on hepatic vitamin E storage is noteworthy in view of the greater use of this fat product in current production of turkeys. Further study is necessary to determine the influence of varying fat sources and levels on the requirement for vitamin E and other possible antioxidant components.

LITERATURE CITED

Marusich, W. L., E. DeRitter, E. F. Ogrinz, J. Keating, M. Mitrovic, and R. H. Bunnell, 1975. Effect of supplemental vitamin E in control of rancidity in poutry meat. Poultry Sci. 54:831-844.

Mecchi, E. P., M. F. Pool, G. A. Behman, M. Hamachi, and A. A. Klose, 1956. The role of tocoperol and content in the comparative stability of chicken and turkey fat. Poultry Sci. 35:1238-1246.

Noll, S. L., M. E. El Halawani, P. E. Waibel, K. A. Janni, and P. Redig, 1987. Management of market turkeys study. Parts 1 and II. Gobbles, Jan. (pp. 16-20) and Feb. (pp. 13-14).

Rethwill, E. E., T. K. Bruin, P. E. Waibel, and P. B. Addis, 1981. Influence of dietary fat source and vitamin E on market stability of turkeys. Poultry Sci. 60:2466-2474.

TABLE 1. Influence of diet fat and vitamin E on TBA reactive substances in stored muscle and on hepatic vitamin E.

Diet Type	Diet Vitamin E[1]	Muscle TBA [2]	Hepatic alpha-tocopherol [3]
Corn-soybean	0	2.8	1.8
	10	1.8	10.0
	300	.14	17.8
Hydrolyzed animal-vegetable fat (8%)	0	3.7	0.46
	10	3.0	0.63
	300	.74	14.7
Soybean oil (8%)	0	1.6	3.5
	10	1.7	3.8
	300	.30	21.4
Animal fat (8%)	0	3.3	1.5
	10	2.6	2.1
	300	.26	17.6

1 Diet vitamin E (IU/kg) provided as dl-alpha tocopheryl acetate

2 TBA reactive substances, ug/g meat (tibial extensor digitorun longus). Unfrozen turkeys were held at 3 C for 3 weeks (Rethwill et al., 1983).

3 mg alpha-tocopherol/g wet tissue

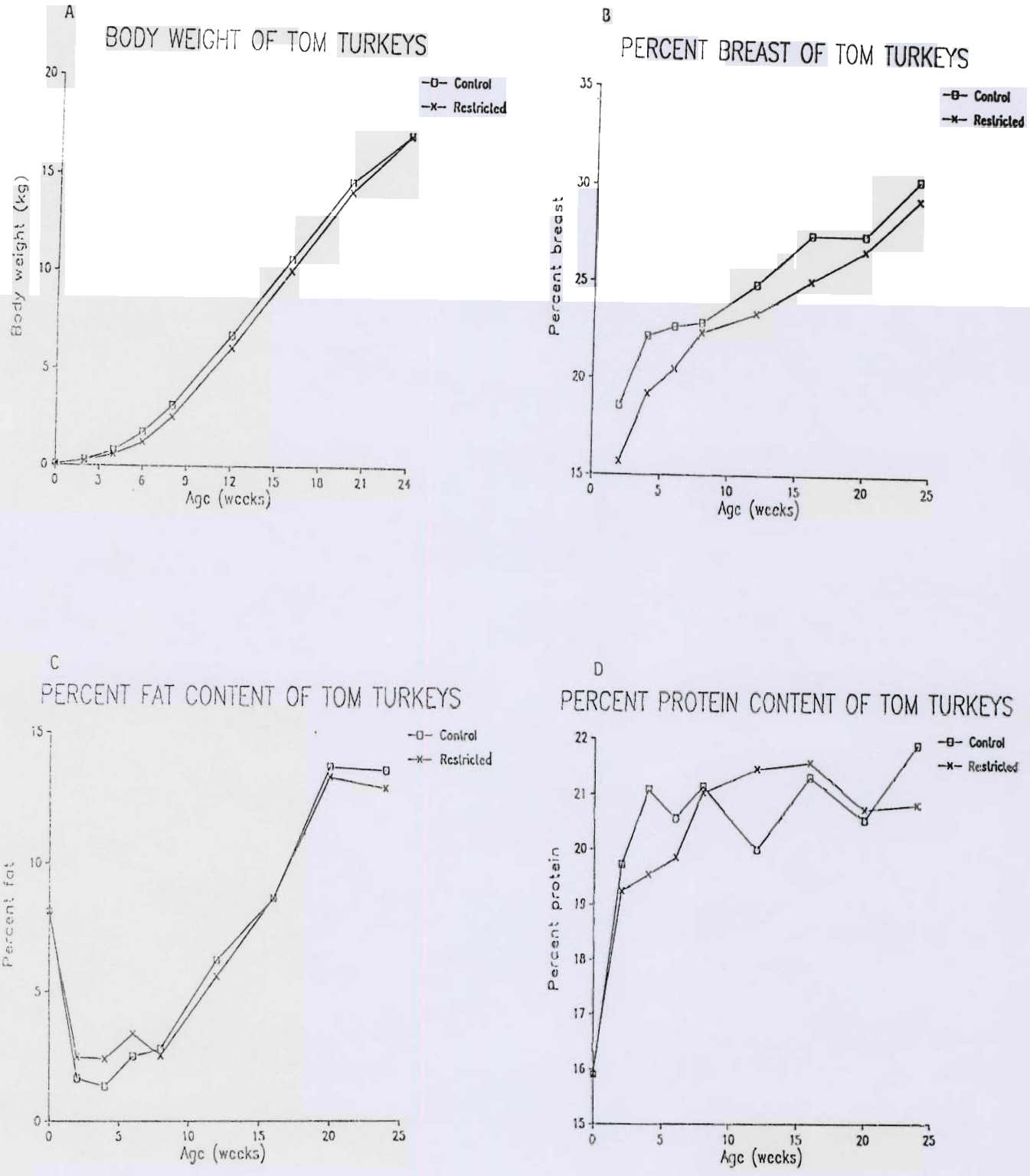


Figure 1. Influence of compensatory growth on body weight (A), percent (B), percent carcass fat (C), and percent carcass protein (D) of tom turkeys.

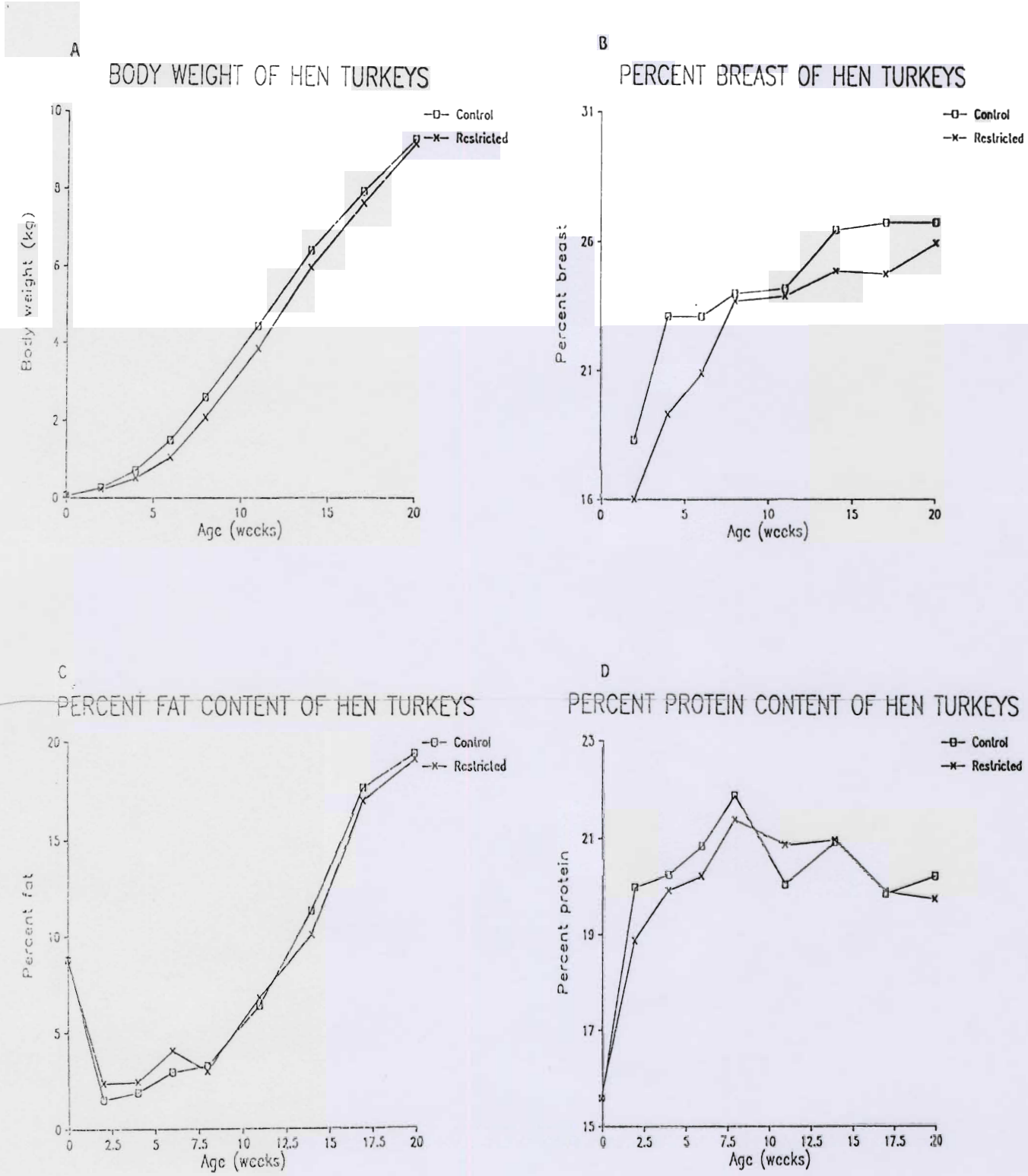
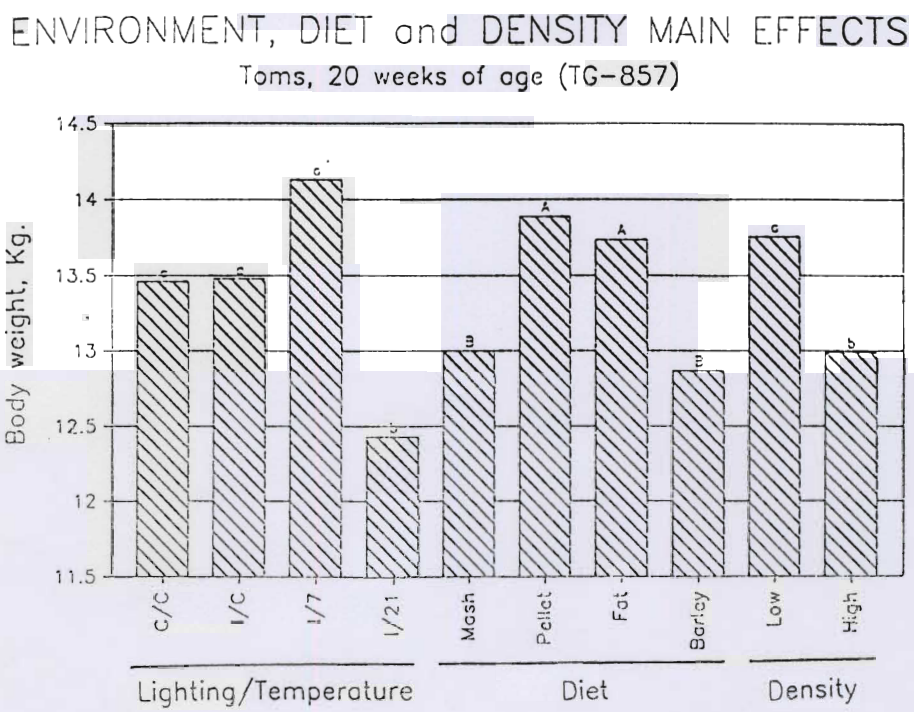


Figure 2. Influence of compensatory growth on body weight (A). percent breast (B), percent carcass fat (C), and percent carcass protein (D) of hen turkeys

A



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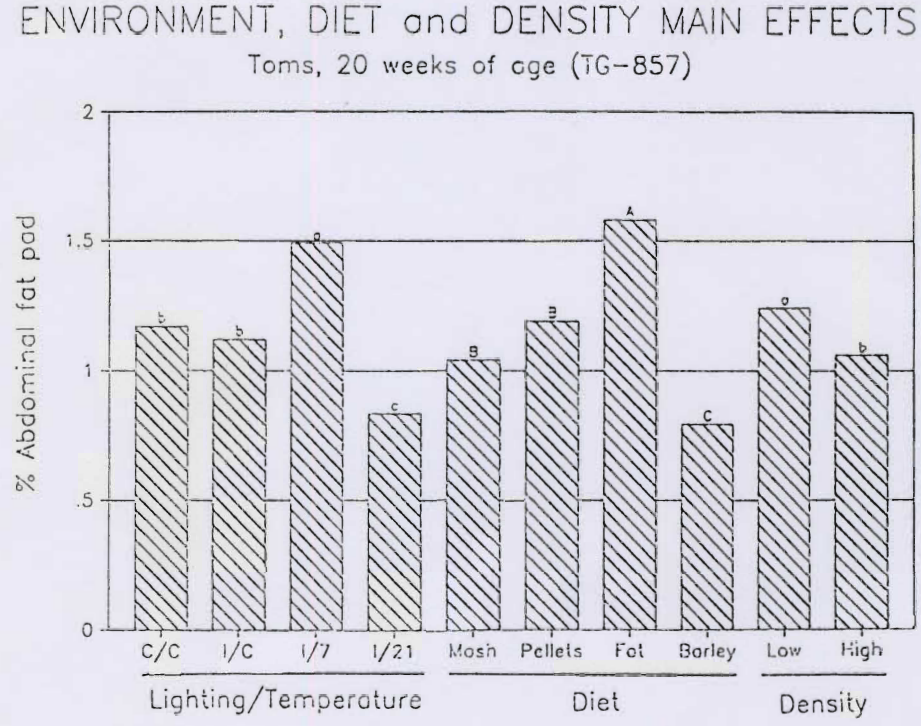
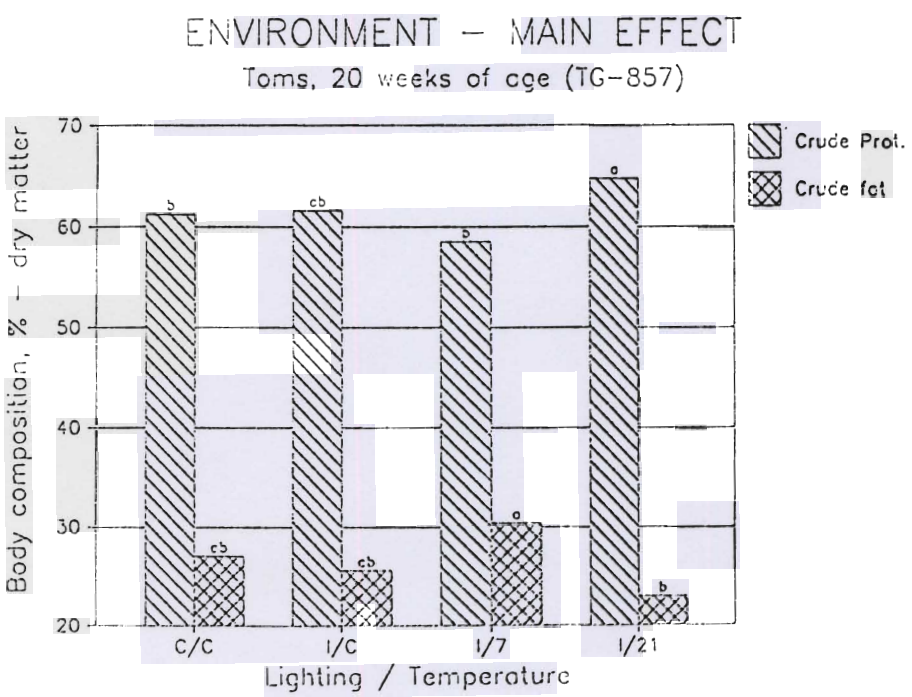


Figure 3. Influence of environment, diet, and bird density main effects on body weight (A) and percent abdominal fat pad (B).

A



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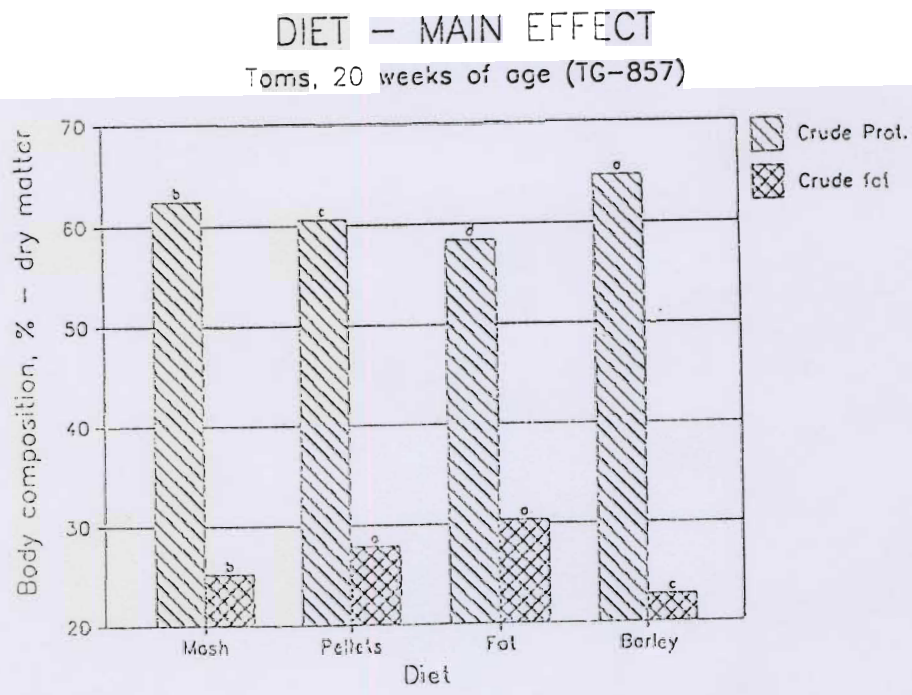

Figure 4. Influence of environment (A) and diet (B) main effects on protein and fat in carcass dry matter.

THE PREDICTION OF CARCASS COMPOSITION IN TURKEYS BY BLOOD LIPIDS

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INTRODUCTION

In broilers, numerous workers have shown a positive genetic correlation between body weight and fat deposition. Also, selection for increased growth rate (heavier body weight) has resulted in excessive amounts of carcass fat at slaughter age. Some of these studies have also shown that different genetic stocks differ in the amount of carcass fat percent at market weight (reviewed by Lilburn, 1987). Several authors have reported that lines selected for leanness also show a significant improvement in feed efficiency with the added potential for enhanced protein deposition and edible protein yield (Lilburn, 1987).

Fatness in chickens is known to be heritable (Ricard and Rouvier, 1967) and thus selection against fatness could be used to develop leaner lines of broilers. Post-mortem measurement of abdominal fat pads of siblings was used by Leclercq *et al.* (1980) to develop lean and fat lines. A different approach, based on the measurement of very-low-density lipoprotein (VLDL) has also been used to develop fat and lean lines of broilers. Plasma VLDL has been shown to be highly correlated (up to .7) with carcass fat (Whitehead and Griffin, 1982). Preliminary data, showing the same relationship may hold for turkeys, have also been reported (Griffin and Whitehead, 1985).

Females contain considerably more fat than males at body weights greater than 3 kg, or at ages greater than 8 weeks (Leeson and Summers, 1980; Bacon and Nestor, 1985). While selection for increased weight is continuing in commercial turkey breeding stocks, age at marketing has decreased rather than the marketing of heavier birds. Thus, carcass composition of market turkeys has not changed drastically with selection for increased body weight. However, in the future, carcass fat content may reach undesirable levels, especially in females, if market weight increases. Lipid (fat) stored in adipose tissue is mainly triacylglyceride (TG). In birds, TG is either absorbed directly from the diet or synthesized from excess energy not needed for maintenance. Transportation of absorbed (exogenous) lipid from the gut, or of *de novo* synthesized lipid (endogenous) to the site of utilization is accomplished by the circulatory system. During transportation, the water insoluble TG is combined with proteins, phospholipids, and cholesterol to form lipoproteins.

Dietary lipoproteins are secreted directly into the portal system in birds and they are referred to as **portomicrons** (Bensadoun and Rothfield, 1972). Portomicrons are the largest and lowest density lipoproteins in the circulatory system. During lipid digestion in the gut, dietary TG is first hydrolyzed to non-esterified fatty acids (NEFA) and monoglycerides (MG) before crossing the intestinal mucosa. A portion of the NEFA and MG is then re-esterified into TG and transported as portomicrons, but a fraction (up to 50%) of the NEFA is not re-esterified, but is transported bound to albumin (Sklan et al., 1984). Both re-esterified and NEFA + MG pass through the liver before entering the extra hepatic circulation, and a large percentage of the absorbed NEFA may be removed during this passage. Portomicrons, however, may be absorbed by the liver only after partial digestion in the peripheral circulation (Bensadoun and Kompiang, 1979). Normally, the concentration of portomicrons is low in the plasma, due in part to their rapid rate of removal ($t^{1/2}$ = 3-5 min; Bensadoun and Kompiang, 1979). When diets with low levels of dietary lipids are fed, portomicron levels can also be expected to be minimal, even when the animals are full fed.

In immature birds, most of the TG is of liver (endogenous) origin and is present in plasma as **very-low-density liproteins** (VLDL). These lipoproteins are smaller than portomicrons but are still very rich in TG. In immature birds fed normal diets and in positive energy balance, most of the circulating TG is in the VLDL fraction. This is especially true when diets low in lipid are fed prior to sample collection, when the concentration of portomicrons is low.

Lipoprotein lipase is the most important enzyme involved in the removal of TG from portomicrons and VLDL. Lipoprotein lipase catalyzes the hydrolysis of TG to glycerol and NEFA. Lipoprotein lipase is found on the endothelial lining of capillary beds of adipose and muscle tissue. The NEFA released in muscle tissue may be oxidized for energy, while NEFA released in adipose tissue may be re-esterified and stored as TG.

Once TG is resynthesized and stored in adipose tissue, it may undergo hydrolysis under periods of energy deprivation and be mobilized as NEFA. Glucagon is thought to be the primary hormone controlling adipose tissue lipid mobilization in birds. During periods of starvation, NEFA may increase three to four fold to levels seen in full fed birds. NEFA are normally present in only small quantities in the plasma, but they have a very short $t^{1/2}$ (Bacon et al., 1970), and thus the flux rate for the NEFA may still be quite large.

The objective of the present work was to see if carcass fatness could be predicted by one or a combination of blood lipid concentration measurements. We specifically chose blood TG as an indicator of lipogenesis and NEFA as an indicator of lipolysis. Both males and females were studied, as were lines differing in growth rate.

MATERIALS AND METHODS

Two experiments were conducted. In Experiment I, 20 week old birds were utilized. These birds were of the RBC-2 strain maintained at the OARDC. This strain was established twenty years ago from elite commercial strains available at that time. In Experiment II, three lines were utilized at 18 weeks of age. These lines were the RBC-2, F (a line selected from the RBC-2 for increased body weight at 16 weeks of age) and N (a current commercial male line). In both experiments, a diet low in fat, based on corn starch, soybean meal and no added feed fat, was fed for one week prior to the experiment. Fat content of this diet was calculated to be about 1%. The birds were housed in an enclosed shed and given 12 hr of lighting per day. The first blood sample was drawn late in the afternoon and the feed then removed just prior to the beginning of the dark period. A second blood sample was drawn just after the light period began 12 hr later. The birds were weighed when the first blood sample was drawn. Plasma was collected and analyzed for total triglyceride and NEFA concentrations using colorimetric procedures. After the second blood sample was drawn, the birds were slaughtered and carcass percent fat (dry matter basis), abdominal fat, leaf fat, and gizzard fat determined.

RESULTS AND DISCUSSION

In Experiment I, the females were smaller, fatter and contained larger fat pads than males (Table 1). No difference existed between sexes for TG-1 and NEFA-1, measured in the blood sample drawn in the afternoon before the dark period began, indicating that lipogenesis and lipolysis did not differ between the sexes under these full fed conditions. However, after an overnight fast, TG-2 was higher in females and NEFA-2 higher in males, indicating males are better able to mobilize lipid than females under these conditions, while females may either recirculate available plasma NEFA as resynthesized TG bearing lipoproteins, or have higher residual levels of TG bearing lipoproteins after mild fasting. Linear correlations were calculated within sex for carcass percent fat, abdominal, gizzard, and leaf fat as dependent variables and TG-1, TG-2, NEFA-1 and NEFA-2 values as independent variables. These correlations are presented in Table 2. From these correlations, it is evident that different relationships exist between fatness and plasma TG and NEFA measurements in males and females. Body weight, TG-1, and TG-2 were of greatest importance in females in predicting carcass percent fat and abdominal fat, while TG-1 and FFA-2 were of greatest importance in males in predicting carcass percent fat and abdominal fat. Also, in males the correlation between fatness measurements and NEFA was strongly positive, while in females it was close to 0. This indicates that fatter males mobilized more NEFA than less fat males while there was no relation between fatness and NEFA mobilization in females.

In Experiment II, the means of the various traits were broken out according to line, sex, and their interaction, and are presented in Table 3. Body weights were smaller in RBC-2, as expected, and males were heavier than females, also as expected. Abdominal fat was lowest in the smaller RBC-2 and not different between the F and N lines. Also as expected, males had less fat than females. An interaction was present for this trait; females of the smaller RBC-2 line exhibited less of an increase than males in comparison to the larger F and N lines. Percent abdominal fat has not been analyzed statistically, but does follow the same trend as abdominal fat pad weight. The concentration of NEFA-1 was higher in the smaller RBC-2 line, but not different between males and females. In contrast, TG-1 was not different between lines, but higher in females than males in all three lines. The NEFA-1 data indicate that the smaller RBC-2 line is either less efficient in its uptake of NEFA liberated from TG carrying lipoproteins, or that there is a higher endogenous lipolysis rate in this line at this age than in the two larger lines. The TG-1 data indicate that females are synthesizing more TG than males. The NEFA-2 data show that the smaller RBC-2 birds maintained higher concentrations of circulating NEFA than the two larger and fatter lines. Also, males had higher values than females in all lines. Thus, both males and smaller birds were better able to mobilize lipid after a mild overnight fast than were females or larger birds. The TG-2 levels were higher in the larger birds and females. Thus, these birds are either recycling NEFA, or less responsive to the mild overnight fast than are the larger birds or males.

Linear correlations across lines and sexes, and both without and with the treatment effects statistically removed (residual correlations) are presented in Table 4 for abdominal fat, 16 wk body weight, and the TG and NEFA measurements. Abdominal fat is highly correlated with its two component parts, leaf and gizzard fat, and thus this discussion will be limited to abdominal fat. Abdominal fat was weakly correlated with body weight in both the total and residual correlations. Abdominal fat was not, however, associated with NEFA-1 either before or after treatment effects were removed from the data. Abdominal fat was negatively correlated with NEFA-2 when treatment effects were not removed, but when these effects were removed (residual correlations), this effect was lost. As discussed above, both males and the RBC-2 line had higher NEFA-2 concentrations and smaller abdominal fat pads. Since one can easily sex turkeys, and work within lines, these data indicate that there is no beneficial effect of using NEFA-1 (measured in turkeys full fed a low fat diet), or NEFA-2 (turkeys fed a low fat diet but fasted overnight), to predict carcass fatness. Abdominal fat was moderately associated with TG-1 and TG-2, even with the treatment effects removed (residual correlations). The correlation with TG-1 was slightly higher, however. This observation is in agreement with Griffin and Whitehead (1985) who measured VLDL rather than TG in full fed turkeys. The residual correlation of TG-1 and body weight was positive and significant. Thus, it is possible that body weight should be included in equations for estimating carcass fatness.

Multiple correlation analyses were conducted across lines, but within sex, with abdominal fat as the dependent variable and body weight, TG-1, TG-2, NEFA-1 and NEFA-2 as dependent variables. For males, r = .736, with only body weight and TG-1 significantly contributing to the correlation coefficient. For females, r = .812, also with body weight and TG-1 significantly contributing to the correlation coefficient. Since the correlation of TG-1 and body weight is positive, these data indicate that selection against TG-1 would tend to select the smaller birds, not a standard industry goal. Thus, if selection against fatness is practiced using TG-1 as the correlated selection trait, it should be applied only on those birds with adequate growth rate, or after selection for growth has been made.

REFERENCES

Bacon, W.L., and K.E. Nestor, 1985. Carcass Composition Changes With Selection for Growth in Turkeys. Ohio Turkey Days, Dept. Series 90:30-35.

Bacon, W., S.P. Touchburn, and M. Topscher, 1970. Clearance Rates and Initial Transfer of the Major Free Fatty Acids in the Growing male Turkey. Comp. Biochem. Physiol. 34:575-578.

Bensadoun, A., and I.P. Kompiang, 1979. Role of Lipoprotein Lipase in Plasma Triglyceride Removal. Fed. Proc. 38:2622-2626.

Bensadoun, A., and A. Rothfield, 1972. The Form of Absorption of Lipids in the Chicken, Gallus domesticus. Proc. Soc. Exp. Biol. and Med. 41:814-817.

Leclercq, B. J.-C. Blum and J.P. Boyer, 1980. Selecting Broilers for Low or High Abdominal fat: Initial Observations. British Poultry Sci. 21:107-113.

Leeson, S. and J.D. Summers, 1980. Production and Carcass Characteristics of the Large Turkey. Poultry Sci. 59:1237-1245.

Lilburn, M.S., 1987. Commercial Consequences of Selecting for Leanness in Poultry. European Symposium on Genetic, Metabolic and Hormonal Basis of Leanness in Domestic Birds Station de Recherches Avicoles Nouzilly 37380 Monnaie France. in press

Ricard, F.H. and R. Rouvier, 1967. Etude de la Composition Anatomique du Poulet. I. Variabilite de la Repartition des Differentes Parties Corporelles chez des Coquelets "bresse-pile). Annls. Zootech. 16:23-29.

Sklan, D., A. Geva, P. Budowsky and S. Hurwitz, 1984. Intestional Absorption and Plasma Transport of Lipids in Chicks and Rats. Comp. Biochem. Physiol. 78A:507-510.

Whitehead, C.C. and H.D. Griffin, 1982. Plasma Lipoprotein Concentration as an Indicator of fatness in Broilers: Effect of age and diet. British Poultry Sci. 23:299-305.

Table 1. Means for body weight, fat measurements, and triacylglyceride (TG) and nonesterified fatty acids (NEFA) in male and female turkeys.

Trait	Males (n = 9)		Females (n = 9)	
	mean	σ	mean	σ
Body wt. (kg,20 wks)	9.39	.91	6.32	.49
% Carcass fat (d.m.)	19.00	5.40	28.50	6.30
Abdominal fat (gm)	22.00	17.00	57.30	28.00
Leaf fat (gm)	12.40	10.50	30.80	15.30
Gizzard fat (gm)	9.66	6.80	26.50	14.80
TG-1 (u equiv/ml)	1.097	.210	1.151	.256
TG-2 (u equiv/ml)	.257	.036	.370	.093
NEFA-1 (u equiv/ml)	.118	.045	.111	.045
NEFA-2 (u equiv/ml)	.232	.062	.193	.048

TG-1, full fed low fat diet
TG-2, after overnight fast
NEFA-1, full fed low fat diet
NEFA-2, after overnight fast

Table 2. Linear correlations among selected traits within sex.

Sex	Independent variable	Dependent variable			
		% Carcass fat	Abdominal fat	Leaf fat	Gizzard fat
Male (n=9)	Body weight	.396	.401	.400	.386
	TG-1	.604	.844**	.845**	.807**
	TG-2	-.015	.186	.179	.190
	NEFA-1	-.481	-.226	-.264	-.158
	NEFA-2	.545	.536	.506	.560
Female (n=9)	Body weight	.411	.830**	.729*	.831**
	TG-1	.595	.747*	.782*	.824**
	TG-2	.590	.720*	.534	.824**
	NEFA-1	-.205	-.365	-.410	-.273
	NEFA-2	-.329	.046	-.008	-.080

TG-1, full fed low fat diet
TG-2, after overnight fast
NEFA-1, full fed low fat diet
NEFA-2, after overnight fast

Table 3. Means of the various traits measured in Experiment II broken down according to line, sex, and their interaction.

Factor	n	Body wt (16 wk) (kg)	Abdominal fat (g)	Abdominal fat (%)	TG-1 (u equiv/ml)	TG-2 (u equiv/ml)	NEFA-1 (u equiv/ml)	NEFA-2 (u equiv/ml)
Line								
R-2	61	6.26[a]	23.2[a]	.37	.845	.263[a]	.140[b]	.389[b]
F	61	9.34[b]	63.6[b]	.67	.930	.394[b]	.108[a]	.381[a]
N	57	9.34[b]	64.6[b]	.69	.916	.333[b]	.119[a]	.319[a]
Sex								
M	90	9.58[b]	35.9[a]	.38	.692[a]	.255[a]	.123	.368[b]
F	89	7.06[a]	65.0[b]	.91	1.102[b]	.405[b]	.122	.317[a]
Interaction (Line X Sex)[1]								
R-2 -M	31	7.40	17.2	.23	.694	.238	.152	.394
-F	30	5.13	29.3	.57	.995	.288	.128	.383
F -M	30	10.71	43.6	.41	.724	.288	.107	.369
-F	31	8.00	81.0	1.01	1.135	.499	.109	.266
N -M	29	10.62	47.2	.44	.657	.239	.111	.340
-F	28	8.05	82.1	1.01	1.175	.427	.128	.289

TG-1, full fed low fat diet
TG-2, after overnight fast
NEFA-1, full fed low fat diet
NEFA-2, after overnight fast

1 Interaction effects were present for abdominal fat and TG-2. These effects are attributed to smaller differences between the sexes within the R-2 line than within the F and N lines.

a,b Means with different superscripts within factors and columns are significantly different ($P \leq .05$)

Table 4. Correlations between abdominal fat, body weight (18 wk) and the triacylglycerol (TG) and nonesterified fatty acid (NEFA) measurements. Above the diagonal are the values before correction for line and sex effects, and below the diagonal are the values after correction for line and sex effects (residual correlations).

	Abdominal fat	Body wt	TG-1	TG-2	NEFA-1	NEFA-2
Abdominal fat	-----	.213*	.524**	.531**	-.080	-.268**
Body wt	.213**	-----	-.223**	-.049	-.114	-.076
TG-1	.406**	.220**	-----	.554**	.143	.002
TG-2	.331**	.123	.387**	-----	-.006	-.377**
NEFA-1	.017	.101	.159*	-.020	-----	.141
NEFA-2	-.073	-.070	.140	-.305**	.117	-----

TG-1, full fed low fat diet
TG-2, after overnight fast
NEFA-1, full fed low fat diet
NEFA-2, after overnight fast

* $P \leq .05$
** $P \leq .01$

VITAMIN D METABOLISM AND SKELETAL DISORDERS

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Vitamin D is an essential nutrient for poultry. Its action is to promote the absorption of calcium from the intestine, and deficiency of vitamin D mimics deficiency of calcium. The disease caused by deficiencies of either nutrient in young animals is rickets. Rickets is characterized by decreased activity in the flock, reluctance of the birds to move when disturbed, lameness, and broken bones. On postmortem examination, the bones are soft, and bend before they break. The cartilaginous growth plate in long bones is frequently thicker than normal (> 2mm).

Dietary supplementation with vitamin D is frequently done as a preventative or therapeutic measure to combat skeletal disorders. This report described two situations in which we have studied the relationship of vitamin D to skeletal diseases.

INTERACTIONS OF VITAMINS A AND D

Vitamin D is often administered to flocks as one ingredient in a multiple vitamin pack. Our work (6), and that of other researchers (10,11), suggests that, at best, this may not be the most effective means to give vitamin D, and, at worst, may have deleterious effects. The effects of vitamin D may be masked or counteracted by other ingredients in the multiple vitamin pack, particularly by vitamin A. We have studied the interaction of vitamins A and D as it relates to skeletal development in the poult, and have investigated the mechanisms of this interaction.

Experiment 1: Diets with different levels of vitamins A and D were fed to tom poults from day of age until 3 1/2 weeks of age. Poults fed a diet deficient in both vitamins developed lameness, growth depression, mortality, and rickets. Poults fed a diet containing the required level of vitamin D (900 ICU/kg) and a high level of vitamin A (400,000 IU/kg) developed rickets similar to those on the basal deficient diet. The abnormalities were characterized by soft bones, decreased bone ash, decreased bone mineral content (as determined by photon absorptiometry), decreased weight gain, and decreased longitudinal bone growth. The abnormalities induced by the high vitamin A diet were not seen when high vitamin D levels (900,000 ICU/kg) were included with high vitamin A (Table 1). This provides evidence that the deleterious effect of high vitamin A may be due to interference with the absorption and/or metabolism of vitamin D, and that administration of excess vitamin D in conjunction with high vitamin A can prevent rickets due to high vitamin A (6).

TABLE 1. Body weight, bone mineral content, percent bone ash and tibia length.

Diet	Body wt (g)	Bone mineral content x 10^3 (g/cm)	% Bone ash	Tibia length (cm)
High vitamin A	312.8[a]	33.7[a]	28.8[a]	7.5[a]
High vitamin D	526.9[b]	100.2[b]	43.2[b]	8.5[b]
High vitamin A+D	596.3[bc]	105.7[b]	47.6[b]	8.8[b]
Control	640.7[c]	105.3[b]	44.0[b]	8.8[b]

Experiment 2: A trial was designed to assess the effect of hypervitaminosis A on the metabolism of vitamin D. Vitamin D must undergo two chemical hydroxylation steps to form the active metabolic, 1,25-dihydroxyvitamin D_3 ($1,25-(OH)_2-D_3$). The first hydroxylation step occurs in the liver and the product is 25-hydroxyvitamin D_3 ($25(OH)-D_3$). This compound has no biological activity, but its level in the blood accurately reflects the amount of native vitamin D_3 which has been absorbed from the intestine. The second hydroxylation occurs in the kidney to form $1,25-(OH)_2-D_3$. This metabolite is the hormonal form of the vitamin which promotes the absorption of calcium from the intestine.

The design of the feeding trial was similar to that of Experiment 1. Poults that were fed a diet containing the NRC recommended level of vitamin D (900 ICU/kg) and a high level of vitamin A (400,000 IU/kg) had signs of vitamin D deficiency, including hypocalcemia. The plasma levels of $25-(OH)-D_3$ (1,2,9) were reduced to about one-third of normal. Similarly, when poults were fed rations high in vitamin D_3 (900,000 ICU/kg), the plasma levels of $25-(OH)-D_3$ were significantly less when a high level of vitamin A was present in the diet than when a normal level of vitamin A was present (Table 3). The most probable cause for this antagonistic effect is that vitamin A interferes with the intestinal absorption of vitamin D.

TABLE 2. Plasma levels of calcium and vitamin D metabolites.

Diet	Calcium (mg/dl)	$25-OH-D_3$ (ng/ml)	$1,25-(OH)_2-D_3$ (pg/ml)
Basal deficient	10.8[ab]	0.35[ab]	30.0[b]
High vitamin A	9.5[a]	0.27[a]	19.5[ab]
High vitamin D	12.4[c]	7.84[d]	12.4[a]
High vitamin A+D	11.5[bc]	4.89[c]	14.8[a]
Control	11.5[b,c]	0.79[b]	17.6[a]

The high level of $1,25-(OH)_2-D_3$ (8) in the poults on the basal deficient and high vitamin A diets due to the influence of parathyroid hormone (PTH). In the face of hypocalcemia, PTH is secreted, and this hormone stimulates the activity of 1-alpha-hydroxylase in the renal tubular epithelial cells. It is this enzyme that converts $25-(OH)_2-D_3$ to $1,25-(OH)_2-D_3$, and the stimulated enzyme converts the majority of its substrate to the product (4).

Experiment 3: The effect of vitamin A on the intestinal absorption of vitamin D was experimentally investigated using isolated duodenal loops. Turkeys were anesthetized, and a portion of intestine was surgically exposed and ligated at both ends to form a sac. Solutions containing vitamin A and radioactively (^3H) labeled vitamin D_3 were infused into the sac, and the absorption of the labeled vitamin D_3 was determined. Poults on a control diet, and those that had received a feed high in vitamin A were tested. In this experimental system, vitamin A did not affect the absorption of vitamin D_3. Conversely, those poults which were fed a ration high in vitamin A seemed more efficient in absorbing vitamin D_3 than those fed a control ration. The apparent paradox between the results of the animal trials and those with the experimental model have yet to be resolved (5).

VITAMIN D AND TIBIAL DYSCHONDROPLASIA

Tibial dyschondroplasia (TD) is a common abnormality of broilers and heavy turkeys, particularly males. The condition frequently is present in lame birds, but it can also be found in birds with a normal stance and gait (13). The cause(s) of TD in the field are unknown, but several factors have been incriminated. These include genetic predisposition, rapid weight gain, high chloride content of the diet, rations low in calcium and high in phosphate, and ingestion of a specific mycotoxin, fusarochromanone (3,7,12).

The fusarochromanone model of TD was used to determine whether plasma levels of 25-OH-D_3 and 1,25-$(OH)_2$-D_3 differ in chicks with TD. Broiler chicks were dosed with fusarochromanone twice daily from days 3-54. Body weight, bone mineral content, and plasma levels of vitamin D metabolies, calcium, and phosphorus were determined at intervals beginning at day 23.

The data demonstrated that the metabolic pathways of vitamin D are intact in experimental TD. They also demonstrate that a defect in mineralization of the cortex of the tibia (and other long bones) is not associated with TD (Table 3).

TABLE 3. Mean values of experimental parameters.

	Age (days)			
	23	33	44	54
Body weight (kg)				
Control	0.50	1.00	1.62	2.09
TD	0.41	0.86	1.38	1.86
BMC/BW				
Control	211.5	231.8	243.7	234.8
TD	190.3	224.4	235.2	227.6
25-OH-D_3 (ng/ml)				
Control	24.3	31.2	33.0	33.4
TD	23.8	25.7	23.1	43.0
1,25-$(OH)_2$-D_3 (pg/ml)				
Control	89.0	125.5	115.3	93.7
TD	75.2	108.9	90.3	77.8
Calcium (mg/dl)				
Control	10.9			10.6
TD	10.8			10.5
Phosphorus (mg/dl)				
Control	5.8			5.4
TD	6.5			6.3

LITERATURE CITED

1. Adams, J.S., J.L Clemens and M.F. Holick, 1981. Silica Sep-Pak preparative chromatography for vitamin D and its metabolites. J. Chromatog., 226:198-201.
2. Dabek, J.T., M. Harkonen, O. Wahlroos and H. Aldercruetz, 1981. Assay for plasma 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 by "high performance" liquid chromatography. Clin. Chem., 27:1346-1351.
3. Lee, Y.-L., C.J. Mirocha, D.J. Schroeder and M.M. Walser, 1985. TDP-1, a toxic component causing tibial dyschondroplasia in broiler chickens, and tricothecenes from Fusarium roseum 'Graminearum. Appl. Environ. Microbiol., 50:102-107.
4. Metz, A.L., J. Skochdopole, M.M. Walser and W.G. Olson, 1987. The interaction of dietary vitamin A and vitamin D in the turkey poult: Metabolic parameters. Submitted for publication.
5. Metz, A.L., C.J. Gross, J.R. Richards and M.M. Walser, 1987. The influence of vitamin A on the intestinal absorption of vitamin D in the turkey poult. Submitted for publication.
6. Metz, A.L., M.M. Walser and W.G. Olson, 1985. The interaction of dietary vitamin A and vitamin D related to skeletal development in the turkey poult. J. Nutrition, 115:929-935.
7. Pathre, S.V., W.B. Gleason, Y.-W. Lee and C.J. Mirocha, 1986. The structure of fusarochromanone: new mycotoxin from Fusarium roseum, "Graminearum". Canad. J. Chem., 64:1308-1311.
8. Reinhardt, T.A., R.L. Horst, J.W. Orf and B.W. Hollis, 1984. A microassay for 1,25-dihydroxy-vitamin D not requiring high performance liquid chromatography: Application to clinical studies. J. Clin. Endocrinol. Metab., 58:91-98.
9. Shepard, R.M., R.L. Horst, A.J. Hamstra and H.F. DeLuca, 1979. Determination of vitamin D and its metabolites in plasma from normal and anephric man. Biochem. J., 182:55-59.
10. Stevens, V.I., R. Blair and C. Riddell, 1983. Dietary levels of fat, calcium, and vitamins A and D_3 as contributary factors to rickets in poults. Poult. Sci., 62:2073-2082.
11. Tang, K.N., G.N. Rowland and J.R. Veltmann, Jr., 1985. Vitamin A toxicity: Comparative changes in bone of the broiler and Leghorn chicks. Avian Dis., 29:416-429.
12. Walser, M.M., N.K. Allen, C.J. Mirocha, G.F. Hanlon and J.A. Newman, 1982. Fusarium-induced osteochondrosis (tibial dyschondroplasia) in chickens. Vet. Pathol., 19:544-550.
13. Walser, M.M., F.L. Cherms and H.E. Dziuk, 1982. Osseous development and tibial dyschondroplasia in five lines of turkeys. Avian Dis., 26:265-271.

THE INHERITANCE OF BODY WEIGHT AND ITS GENETIC RELATIONSHIPS WITH CARCASS COMPOSITION AND SKELETAL CHARACTERISTICS IN TURKEYS

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INTRODUCTION

A genetic basis for skeletal problems in meat type poultry has clearly been documented by studies showing that: 1) leg problems in broilers are highly heritable; 2) different broiler and turkey strains differ considerably in the incidence and severity of leg problems; 3) broilers respond to selection for decreased incidence of leg problems; 4) selection for 16-week body weight is associated with increased leg problems in turkeys; and 5) selection for increased shank width (leg bone) is associated with increased walking ability in turkeys (see Havenstein et al., 1988a for references and a more detailed discussion of these points).

Commercial breeders of broiler chickens and turkeys have placed their primary selection emphasis on heavier body weight, and wider breast conformation at market weight. Some emphasis has also been placed on livability, feed efficiency, freedom from leg problems and other defects, and improved reproductive efficiency. The primary emphasis for selection on body weight and breast conformation has resulted in greater increases in breast muscle than leg muscle and skeletal (bone) tissues. Because of this disproportionate change in body parts, leg and skeletal problems have become a major production problem with fast-growing commercial lines of broilers and turkeys.

In broilers, numerous workers have shown a positive genetic correlation between body weight and fat deposition. Also selection for increased growth rate (heavier body weight) has resulted in excessive amounts of carcass fat at slaughter age. Some of these studies have also shown that different genetic stocks differ in the amount of fat percent at market weight.

Several investigators have shown that the amount of body fat has increased in turkeys with selection for growth rate, but is not yet at a level where it is considered to be an economic problem. It is a concern, however, that fatness of turkeys is currently increasing with growth rate selection and that the commercial turkey may accrue the over fat problem currently present in commercial broilers as this selection continues (see Havenstein et al., 1988b, for references and a more detailed discussion).

Selection for increased growth rate in turkeys has led to disproportionate (and commercially desirable) increases in the relative amount of breast muscle, and this change in body

proportions has brought about at least part of the leg weakness problem currently present in the turkey industry (Nestor et al., 1985). Selection for increased growth rate may also be associated with changes in carcass composition, such as fatness.

A study was therefore designed and conducted to estimate the genetic relationships of various skeletal and carcass traits in growing turkeys.

MATERIALS AND METHODS

Data were collected at 16 weeks of age on 1088 fully pedigreed individuals (504 females and 584 males) of the randombred (RBC-2) line of turkeys maintained at OARDC. The RBC-2 line was formed in 1966 from reciprocal crosses of two commercial strains. The RBC-2 line has been maintained with a paired mating system of 36 males and 36 females each generation until the present study when 36 males were mated with 180 unrelated females (5 for each male). Two males and 12 females failed to reproduce, so the present study is based on progeny from 34 males and 168 females.

Two hatches placed 2 weeks apart were pedigree wing-banded and grown sexes intermingled in an enclosed shed until 8 weeks of age, when they were moved to a range until 16 weeks of age. Lighting was continuous the first four weeks, and then natural day length thereafter. A five-ration feeding system with declining levels of protein was fed based on the schedule for males.

During the 16th week of age, all birds were sacrificed over a 5-day period, after an overnight fast. Prior to fast, all individuals were weighed (BW), measured for shank width (SW) at the dewclaw, and the males rated for walking ability (WA; 1 to 5 with 1 excellent and 5 either extreme lateral deviation and/or difficulty in walking).

After fasting, the birds were killed and leaf fat (LF) and abdominal fat (AF; leaf fat plus abdominal fat) removed and weighed. The carcass was then cut in half and one half frozen for later dissection and the other half saved for carcass composition analysis after grinding. Percent moisture (%M) was determined by freze-drying and the dried carcass sample then reground for subsequent determination of percent ash (%A; 600 C for 4 hr) percent fat (%F; chloroform:methanol, 2:1 extraction) and percent protein (%P; Kjeldahl N X 6.25).

On the carcass half saved for dissection, thigh muscles (TM) were removed and weighed as were drum muscles (DM). The breast muscles (BM) were also removed and weighed. The thigh (femur), drum (tibiotarsus) and shank (tarsometatarsus) were weighed immediately after separation from the muscles and these weights are referred to as rough cleaned thigh (RCT), drum (RCD), and shank (RCS) weights. The shank and drum bones were retained and further cleaned by boiling 15 min in water and removing all soft adhering tissue. The cleaned drum (CD) and shank (CS) weights were then

determined. The cleaned drum and shank bones were then extracted for fat (chlorform:methanol, 2:1) and dried at 100 C for 24 hr and weighed to determine fat free drum (FFD) and shank (FFS) weights. Shank length (SL) and drum length (DL) were also determined.

Density of the shank and drum bones was then determined using ^{125}I photon absorption. Density was determined at 40 and 60 percent of bone length and will be referred to as D-40 and D-60 for drums and S-40 and S-60 for shanks.

The data were analyzed and variance and covariance components were estimated. Heritabilities (h^2's), the genetic (r_G) and phenotypic (r_P) correlations were estimated within sex. These analyses are described in detail by Havenstein et al., 1988a and b.

RESULTS AND DISCUSSION

The overall means and standard deviations for the within sex analyses for BW, SW, SL, all of the muscle and fat weights, and the muscle and fat percentages are given in Table 1. Males had higher ($P \leq .05$) mean values than females for BW, SW, SL, BM, DM, %P, %A and %M. However, females had significantly larger mean values than males for all fat and muscle measures when expressed as a percentage of body weight. These observations are consistent with most carcass composition studies, where males generally have larger absolute values for body part and muscle weights, but females generally have larger values for body fat and percentage muscle measurements (see Havenstein et al., 1988 a and b for references and additional discussion).

The overall means and standard deviations for the within sex analyses for BW and the skeletal characteristics are presented in Table 2. Males had larger ($P \leq .05$) mean values for all of these traits, as has been found by others.

Heritability estimates (h^2) for BW and the carcass characteristics are summarized in Table 3. The h^2 estimates for the muscle and fat characteristics show a similar pattern to those for the skeletal characteristics given in Table 4, in that the estimates from the sire components of variance (σ^2_s) from the male data are generally higher than those from the female data. However, h^2 estimates from the dam components of variance (σ^2_D) were higher in females than males. Thus, we conclude that the expression of genetic variation for the same "trait" at the same age is influenced by the sex of the individuals. Therefore, inclusion of "traits" in genetic selection programs should be separate for each sex so as to maximize the improvement of overall genetic merit.

Heritability estimates (h^2) for BW and the skeletal characteristics, estimated from the sire (σ^2_s) and dam (σ^2_D) components of variance, are summarized in Table 4. As has been reported by others (see Havenstein et al., 1988a for references,

the h^2's for most of the skeletal characteristics are relatively high. The exception to this is the h^2 for WA (walking ability) which was relatively low (.06 $\pm$ .08). Since WA was estimated subjectively and the incidence of leg problems is quite low in the RBC-2 line used in this study, this observation is not suprising. Because of the low incidence of leg problems in females, WA was not estimated in them.

Genetic correlation estimates of BW with the muscle weight measurements tended to be high (range .47 to 1.05) in both sexes.

Genetic correlation estimates of BW with all fat measures were positive in both sexes. Leaf fat showed the lowest genetic correlations with BW (.10 for females; .27 for males). Abdominal fat was slightly (.30 for females and .32 for males) and percent carcass fat considerably more highly correlated (.88 for females and .50 for males) with BW than leaf fat. It is apparent that if one were selecting against fatness, the best information would be supplied by percent carcass fat. On the other hand, from a practical point of view, this measurement is relatively difficult and expensive to perform as a routine selection criterion for a commercial turkey breeding program. Also, leaf fat, abdominal fat and percent carcass fat were highly correlated genetically ($r_G \geq$ .95) and they are, therefore, equally good genetic estimates of fatness. However, because all of the measures used in this study would require the use of sacrificed sibs to obtain the selection data, a better non-destructive direct genetically correlated estimate of fatness is needed for practical application of selection for (or against) carcass fatness. It is certain, however, based on these data as well as those data of others (see Havenstein et al., 1988) that continued selection by turkey breeders for BW and breast conformation score as currently practiced will eventually result in excessively fat turkeys, just as it has with broiler chickens. Also, if heavier market weights are economically justified and become a reality, as in the broiler chicken, an increasingly fatter market turkey will result.

The genetic correlations between BW and the various bone weight measurements were extremely high ($\geq$ .75), and the rough cleaned bone weights were just as highly correlated with BW as were the cleaned and fat-free bone weights. BW was correlated to a more moderate degree with shank and drum density scores (r_G = .23 to .79). These relationships were consistently higher for males than females. The r_G of SW with BW is relatively low (males = .33, females = .47) indicating that selection for BW alone might not increase SW enough to support the increased body weight. Body weight was more highly correlated with SL and DL (.53 and .84 for SL from males and females; .55 and 1.10 for DL from males and females). Also, SW was poorly correlated with SL and DL (.32 and .10 for SL from males and females; and .18 and .18 for DL for males and females) and moderately correlated with drum and shank density measurements. Thus selection for BW alone in turkeys would be expected to maintain relative bone length better than bone width, but could be expected to result in a proportionate

decrease in both length and width, but especially in bone width.

The low to moderate r_G estimates between BW and several skeletal characteristics such as bone weights, bone lengths and bone density scores, indicate that selection for BW alone would result in disproportionately low increases in skeletal characteristics in comparison to changes brought about in BW. Therefore, selection for BW or BW and some measure of brest conformation may lead to less than optimal increases in the skeletal support system, thereby leading to genetically induced leg problems. These and other data from OARDC indicate that turkey breeders should use a balanced selection approach which includes some measure of the skeletal support system. It appears that SW and SL and possibly the RCS could provide this measurement.

REFERENCES

Havenstein, G.B., K.E. Nestor, V.D. Toelle, and W.L. Bacon, 1988a. Estimates of Genetic Parameters in Turkeys: 1. Body Weight and Skeletal Characteristics. Poultry Science, prepared for publication.

Havenstein, G.B., V.D. Toelle, K.E. Nestor, and W.L. Bacon, 1988b. Estimates of Genetic Parameters in Turkeys: 2. Body Weight and Carcass Characteristics. Poultry Science, prepared for publication.

Nestor, K.E., W.L. Bacon, Y.M. Saif and P.A. Renner, 1985. The Influence of Genetic Increases in Shank Width on Body Weight, Walking Ability, and Reproduction of Turkeys. Poultry Sci. 64:2248-2255.

Table 1. Overall means and standard deviations (σ) for the within sex analyses for body weight, the muscle and fat weights, and muscle and fat percentages.

Trait[1]		Males		Females	
		mean	σ	mean	σ
BW	(kg)	7.34	0.56	5.25	0.42
BM	(gm)	665.0	73.2	489.0	56.8
TM	(gm)	347.2	41.2	262.9	32.6
DM	(gm)	260.7	29.8	200.3	22.8
LF	(gm)	3.0	3.1	7.9	7.2
AF	(gm)	6.5	5.4	15.4	12.7
%F		13.0	3.0	17.9	4.2
%A		12.6	2.5	11.8	2.5
%P		68.0	3.5	63.9	4.3
%M		70.8	1.8	69.0	1.8
%BM		9.0	0.6	9.3	0.8
%TM		4.7	0.4	5.0	0.4
%DM		3.5	0.3	3.8	0.3
%LF		0.04	0.04	0.15	0.13
%AF		0.09	0.07	0.29	0.23

1 BW = body weight; BM, DM and TM = breast muscle, drum muscle and thigh muscle; LF and AF = leaf fat and abdominal fat; %F, %A, %P, %M, = percentages of fat, ash and protein (dry basis), and moisture of ground carcass; %BM, %TM and %DM = percentages of breast, thigh and drum muscle of body weight; %LF and %AF = percentages of leaf and abdominal fat of body weight.

Table 2. Overall means and standard deviations (σ) for the within sex analyses for body weight and skeletal measurements.

Trait[1]		Males		Females	
		mean	σ	mean	σ
BW	(kg)	7.34	0.56	5.25	0.42
SW	(mm)	13.3	0.5	11.8	0.5
SL	(cm)	15.6	0.5	12.8	0.5
DL	(cm)	20.0	0.6	17.8	0.6
RCT	(gm)	63.3	5.6	36.8	4.6
RCD	(gm)	100.3	9.1	58.8	6.4
RCS	(gm)	78.0	6.2	46.2	5.2
CD	(gm)	53.8	4.7	32.4	3.3
CS	(gm)	29.3	2.7	16.1	1.7
FFD	(gm)	26.4	2.5	17.1	1.6
FFS	(gm)	15.1	1.2	9.1	0.8
D-40		51.3	5.4	39.1	3.5
D-60		56.3	5.9	42.0	3.8
S-40		37.1	3.3	28.6	2.5
S-60		40.1	3.1	32.4	2.5
WA		1.67	0.66	-	-

1 BW = body weight; SW, SL and DL = shank width, shank length and drum length; RCT, RCD and RCS = rough cleaned weights of thigh, drum and shank; CD and CS = cleaned weights of the drum and shank; FFD and FFS = fat-free weights of the drum and shank; D-40, D-60, S-40 and S-60 = relative density of the drum or shank at 40 and 60 percent of the length of the bone from the proximal end; WA = walking ability (1 = normal to 5 = extreme difficulty in walking).

Table 3. Heritability (h^2) estimates from the sire (σ^2_S) and dam (σ^2_D) components of variance within sex for body weight, the muscle and fat weights, and muscle and fat percentages.

Trait[1]	Males				Females			
	σ^2_S		σ^2_D		σ^2_S		σ^2_D	
	h^2	s.e.	h^2	s.e.	h^2	s.e.	h^2	s.e.
BW	.60	.19	.63	.17	.23	.14	1.22	.20
SW	.47	.17	.48	.17	.55	.20	.68	.20
SL	.54	.19	.58	.17	.43	.18	.06	.18
BM	.35	.15	.87	.18	.08	.10	.91	.20
TM	.17	.11	.77	.18	.12	.11	.80	.20
DM	.44	.17	.39	.17	.30	.15	.84	.20
LF	.51	.18	.57	.17	.13	.11	.78	.20
AF	.55	.19	.61	.17	.21	.13	.92	.20
%F	.24	.12	.60	.17	.06	.10	.68	.20
%A	[2]		.19	.16	[2]		.18	.18
%P	.20	.12	.31	.16	.07	.10	.61	.20
%M	.11	.09	.08	.15	[2]		.80	.20
%BM	.23	.13	.72	.18	.19	.13	.20	.19
%TM	.00	.07	.68	.18	.07	.10	.48	.19
%DM	.36	.15	.38	.17	.04	.09	.50	.19
%LF	.51	.18	.59	.17	.14	.12	.71	.20
%AF	.56	.19	.64	.17	.21	.13	.87	.20

1 BW = body weight; BM, DM and TM = breast muscle, drum muscle and thigh muscle; LF and AF = leaf fat and abdominal fat; %F, %A, %P, %M, = percentages of fat, ash and protein (dry basis), and moisture of ground carcass; %BM, %TM and %DM = percentages of breast, thigh and drum muscle of body weight; %LF and %AF = percentages of leaf and abdominal fat of body weight.

2 non-estimable due to a negative variance component estimate.

Table 4. Heritability (h^2) estimates from the sire (σ^2_s) and dam (σ^2_d) components of variance within sex for body weight and skeletal measurements.

	Males				Females			
Trait[1]	σ^2_s		σ^2_d		σ^2_s		σ^2_d	
	h^2	s.e.	h^2	s.e.	h^2	s.e.	h^2	s.e.
BW	.60	.19	.63	.17	.23	.14	1.22	.20
SW	.47	.17	.48	.17	.54	.20	.68	.20
SL	.54	.19	.58	.17	.43	.18	.05	.18
DL	.60	.20	.59	.17	.66	.22	.09	.18
RCT	.60	.20	.59	.17	[2]		1.67	.19
RCD	.57	.19	.21	.16	.09	.10	.37	.19
RCS	.69	.21	.54	.17	.28	.15	[2]	
CD	.76	.22	.48	.17	.37	.17	.38	.19
CS	.45	.17	.23	.16	.30	.15	.20	.19
FFD	.93	.25	.43	.17	.44	.18	.60	.20
FFS	.82	.23	.46	.17	.45	.18	.43	.19
S-40	.69	.21	.39	.17	.28	.15	.78	.20
S-60	.55	.19	.24	.16	.31	.15	.76	.20
D-40	.80	.23	.58	.17	.68	.22	.49	.19
D-60	.92	.25	.46	.17	.34	.16	.79	.20
WA	.06	.08	.23	.16	-		-	

1 BW = body weight; SW, SL and DL = shank width, shank length and drum length; RCT, RCD and RCS = rough cleaned weights of thigh, drum and shank; CD and CS = cleaned weights of the drum and shank; FFD and FFS = fat-free weights of the drum and shank; D-40, D-60, S-40 and S-60 = relative density of the drum or shank at 40 and 60 percent of the length of the bone from the proximal end; WA = walking ability (1 = normal to 5 = extreme difficulty in walking).

2 non-estimable due to a negative variance component estimate.

NEW DIAGNOSTIC TESTS FOR CHLAMYDIOSIS

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INTRODUCTION

Avian chlamydiosis (AC) is a naturally occurring, systemic, contagious disease of birds caused by Chlamydia psittaci. This bacteria is a obligate intracellular parasite which infects a wide host range including mammals, birds and arthropods. The disease caused by this organism has been referred to as PSITTACOSIS (PARROT FEVER) in psittacine birds and ORNITHOSIS in domestic birds and wild waterfowl.

Acute avian chlamydiosis (AC) in turkeys and ducks has periodically resulted in serious agricultural losses when sporadic epiornitics (avian outbreaks) have affected large concentrations of birds in commercial establishments. In the USA agricultural losses from turkey mortality and meat and egg condemnations reached a peak during 1954-56, when turkeys in Texas, Oregon, New Jersey, and California were involved in a series of lethal epiornitics. The disease reappeared in Texas in 1974-75. While mortality and debilitation of diseased turkeys were severe, agricultural losses were minimized because of prompt recognition of the disease by Federal and State regulatory agencies followed by obligatory quarantine and treatment of the diseased flocks.

A sharp rise in chlamydia in US citizens occurred in 1954 when 563 cases were reported, 200 (35.5%) of which were associated with processing turkeys and 33 (5.9%) were related to contact with chickens. During a three month period in 1974 when diseased turkeys raised in Texas were being sent to slaughtering plants in Texas, Nebraska, and Missouri, 120 processing plant employees developed illness compatable with chlamydia. Chlamydial epiornitics of mild virulence occurred in California in 1980 and 1981, but no human infections were associated with them.

In Minnesota in 1986 there was an outbreak of AC involving approximately 12 flocks, 3 processing plants, and over 175 human cases were reported. The economic burden of this outbreak was substantial when one accounts for morbidity and mortality in the affected flocks, medication costs, losses due to closing plants and returning suspect flocks to farms, loss of time due to illness and medical costs of affected plant workers.

Over the years a number of laboratory tests have been used for the diagnosis of avian chlamydiosis[2, 3, 4, 5, 6, 7, 8, 10, 11]. The methods in current use include Latex Agglutination (LA)[6], Direct Complement Fixation (DCF)[5], culture of fecal and tissue samples,

and stained smears of tissues and conjunctival or nasal exudate[4,8]. There are advantages and disadvantages to each of the above mentioned tests. Usually more than one test is used along with the clinical history to determine the status of a flock.

Grimes in a recent publication comparing the LA and DCF tests concluded that LA was significantly less sensitive than DCF in detecting antibody activity in birds that were culture positive, suggesting that detection of antibody by LA alone was highly indicative of a current or very recent infection. He found a 72.5% agreement in fourfold increase in paired sera titers between the two tests. He speculated that when there was a lack of correlation between LA and DCF titers it may be due to different classes of immunoglobulins present at various stages of infection or recovery, which in turn react differently in the two test methods. LA titers decreased more rapidly than DCF with a comparative sensitivity of 39.1% and specificity of 98.8%[6].

The disadvantages relating to isolation of the organism are the time required to obtain results and the fact that only specialized laboratories have the capabilities for culturing. Additionally, Andersen has found there is a loss of viability of the organism from both freezing and 24 hr. storage (at 4C) of the sample to be tested[1].

The major drawback to stained smears is its low sensitivity as it only identifies elementary bodies. A negative result does not exclude the presence of chlamydia.

ELISA antibody tests are being used extensively as a diagnostic tool for many diseases in a wide variety of hosts. This test has a number of advantages over other diagnostic tests, such as its increased sensitivity, ability to obtain a titer from a single serum dilution, and the very small sample size required to perform the test (0.005ml)[12]. The use of ELISA for the detection of Chlamydial antibodies have been reported in humans[2], ducks[3], turkeys[7], and free-flying birds[11].

Recently a group of workers in Maryland have reported on a Dot-Immunobinding Technique using monoclonal antibody for the detection of Chlamydia trachomatis in clinical specimens from human patients[9]. The key element in the test is its reliance on nitrocellulose membranes to which the organism becomes firmly bound. The membrane binds 90% of the antigen compared to plastic plates which bind only 25% of the antigen. This increased antigen binding improves the sensitivity of the test allowing it to detect 75 picagrams (approx 100 organisms). Other advantages of this test include its ability to detect non viable organisms, and its adaptibility as a rapid diagnostic test.

MATERIALS AND METHODS

OBJECTIVES

1. Develop an Indirect Enzyme Linked Immunobsorbent Assay (ELISA) test for the detection of Chlamydia antibodies in domestic avian sera.

2. Compare the sensitivity and specificity of the ELISA test with the currently used Direct Complement Fixation (DCF) and Latex Agglutination (LA) tests.

3. Develop a Dot-Immunobinding Technique (DIBT) using monoclonal antibodies for detection of chlamydia antigen in avian fecal samples.

4. Compare the DIBT to conventional antigen capture techniques.

METHODS

Infection of Turkeys.

Turkeys will be infected with the Texas Turkey (TT3) isolate of chlamydia in isolation pens by Dr. A.A. Andersen, NADL, Ames, IA. Serum and fecal samples will be taken from infected birds pre infection and at 2, 4, 7, 14, 21, 28, 42 and 56 days post infection.

Serum and fecal samples.

Serum and fecal samples will be obtained from experimentally infected turkeys as outlined above.

ELISA technique.

1. ELISA plates precoated with Chlamydia trachomatis provided by Whittaker M. A. Biologics.

2. Add 100ul test serum (1/1600)/well, and incubate 30min at 37C.

3. Wash 3 times with PBS Tween.

4. Add 100ul of phosphatase labelled goat anti-turkey IgG, and incubate 30 min at 37C.

5. Wash 3 times in PBS Tween.

6. Incubate 15 min at 37C in 100ul of SIGMA 104 Phosphatase in 10% Diethanalomine Buffer pH 9.6.

7. Stop by adding 25ul of 5N NaOH

8. Read at 405 nm.

Dot-Immunobinding Technique.

1. Rehydrate nitrocellulose strip in Distilled H_2O followed by Tris Buffered Saline (TBS). Blot with filter paper.

2. Apply samples to nitrocellulose (5ul) and allow to dry for 20 min.

3. Block open receptors by incubating at room temp. (RT) in 0.5% whey in TBS for 20 min.

4. Apply prepared polyclonal antibody. Incubate at RT for 20 min.

5. Wash 3 times in TBS.

6. Apply goat anti-mouse IgG labelled with biotin. RT 20 min

7. Wash 3 times TBS.

8. Apply peroxidase labelled streptavidin. RT 20 min.

9. Wash 2 times TBS and 1 time dist. H_2O.

10. Add substrate (4-chloro-1napthol + H_2O_2).

11. Stop in distilled H_2O and read.

Monoclonal antibodies.

Monoclonal antibodies were provided by Dr. A. A. Andersen, NADL, Ames. IA. Five of the monoclonal antibodies were specific for the turkey strain of Chlamydia psittaci and one was a group reactive antibody.

RESULTS

The results of the above tests will be presented and discussed.

REFERENCES

1. Andersen, A.A. Personal communication.

2. Evans, R.T., and D. Taylor-Robinson. Development and evaluation of enzyme-linked immunosorbent assay (ELISA), using chlamydial group ntigen, to detect antibodies to Chlamydia trachomatis. J. Clin. Pathol. 35:1122-28. 1982.

3. Evans, R.T., W.S.K. Chalmers, P.R. Woolcock, H. Farmer, and D. Taylor-Robinson. An enzyme-linked immunosorbent assay (ELISA) for the detection of chlamydial antibodies in duck sera. Avian Path. 12:117-124. 1983.

4. Gerlach, H. Chlamydia. 1st edit. In: Clinical Avian Medicine and Surgery. Eds. Harrison & Harrison. pp 457-463. 1986.

5. Grimes, J.E., L.C. Grumbles, and R.W. Moore. Complement-fixation and hemagglutination antigens from a chlamydial (ornithosis) agent grown in cell cultures. Can. J. Comp. Med. 34:256-260. 1970.

6. Grimes, J.E. Chlamydia psittaci latex agglutination antigen for rapid detection of antibody activity in avian sera: Comparison with direct complement fixation and isolation results. Avian Dis.30:60-66. 1986.

7. Lang, G.H. La Chlamydiose du dindon. Diagnostic serologique par la methode ELISA. Rec. Med. Vet. 160:763-771. 1984.

8. Paster, M.B. Chlamydiosis: A review of the current literature, treatment, and control. Proc. 1985 An. Mtg. AAV Boulder Colorada. June 4-7. 215-247.

9. Patel, J.D., J.M. Joseph, and W.A. Falkler Jr. Direct detection of Chlamydia trachomitis in clinical specimens by the Dot-Immunobinding technique using monoclonal antibody. Submitted to: J. Clin. Micro.

10. Petrak, M.L. The use of an Immunoperoxidase-monoclonal antibody procedure in the diagnosis of avian chlamydiosis. Proc. 1985 An. Mtg. AAV Boulder, Colorado. June 4-9. 265-266.

11. Ruppanner, R., D.E. Behymer, W.J. DeLong III, C.E. Franti, and T.E. Schultz. Enzyme-immunoassay of chlamydia in birds. Avian Dis. 28: 608-615. 1984.

12. Snyder, D.B. Latest developments in the Enzyme-linked immunosorbent assay (ELISA). Avian Dis. 30: 19-23. 1986

AVIAN CHOLERA - NEW APPROACH FOR SUBUNIT VACCINE

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Avian cholera (pasteurellosis) is one of the most significant causes of economic loss in the U.S. turkey industry with annual cost estimated to be in excess of $200 million. Different somatic serotypes of type A Pasteurella multocida are involved in the genesis of avian cholera. The great need for effective prophylaxis is reflected by excellent sales of commercial bacterins and attenuated live vaccines. However, efficacy data and epidemiologic data available for these vaccines indicate that they are not totally effective in preventing the disease.

In the 1970s, Heddleston, Rebers and their associates (5,8) stimulated great interest when they demonstrated that a bacterin prepared from in vivo propagated P. multocida induced cross-immunity against both homologous and heterologous serotypes. In contrast, a bacterin prepared from in vitro propagated P. multocida induced a serotype-specific immunity only against the homologous serotype. Furthermore (4,6), they demonstrated that turkeys that survived exposure to a virulent strain of P. multocida or vaccinated with an attenuated live vaccine also showed cross-immunity against both homologous and heterologous serotypes. Particularly important was the finding that the IgG antibody was associated with cross-immunity (7).

In the 1980s, elegant experiments by Brogden and Rimler (1,2,3,9,10) showed that the immunogen which was responsible for cross-immunity was present in the outer membrane vesicles of the bacteria and termed it cross-protection factor (CPF). Subsequent studies performed primarily in turkeys showed that the CPF was indeed responsible for cross-immunity against both homologous and heterologous serotypes of P. multocida. The authors proposed that the host environment influenced the expression of CPF by the bacteria. Experiments in our laboratory have confirmed these data, and in addition, demonstrated that the CU and M9 live attenuated vaccine strains also contained the CPF.

There are strong reasons that indicate that CPF could be a promising candidate for a subunit vaccine. In the past, isolation and characterization of the CPF have been difficult. We have used a new strategy for identifying the CPF. Our approach has been to produce experimental cholera in turkeys, obtain convalescent sera which are protective, and identify the antigens recognized by the serum in Western blots.

Results demonstrated that two immunodominant antigens in the outer-membrane-enriched fractions of in vivo passaged P. multocida A:3 are recognized by antibodies in convalescent serum and sera from turkeys immunized with the CU and M9 live vaccines. These antigens identified as 94K and 56K are present in high concentrations in in vivo passaged CU, M9 and reference serotype 12 strains of P. multocida. We intend to isolate these 94K and 56K antigens by immunoaffinity chromatography and prove that they are indeed the CPF which is responsible for cross-immunity.

LITERATURE CITED

1. Brogden, K.A. and R.B. Rimler, 1982. Lysates of turkey-grown Pasteurella multocida: Examination of vaccine preparations by electron microscopy. Am. J. Vet. Res. 43:304-309.
2. Brogden, K.A. and R.B. Rimler, 1982. Lysates of turkey-grown Pasteurella multocida: Partial solubilization of the cross-protection factor(s). Am. J. Vet. Res. 43:1781-1785.
3. Brogden, K.A. and R.B. Rimler, 1983. Lysates of turkey-grown Pasteurella multocida: Effects of solubilizing agents on the immunologic properties of membrane vesicles. Am. J. Vet. Res. 44:428-432.
4. Heddleston, K.L., J.E. Gallagher, and P.A. Rebers, 1970. Fowl cholera: Immune response in turkeys. Avian Dis. 14:626-635.
5. Heddleston, K.L. and P.A. Rebers, 1972. Fowl cholera: Cross-immunity induced in turkeys with formalin-killed in-vivo propagated Pasteurella multocida. Avian Dis. 16:578-586.
6. Heddleston, K.L., P.A. Rebers, and G. Wessman, 1975. Fowl cholera: Immunologic and serologic response in turkeys to live Pasteurella multocida vaccine administered in the drinking water. Poultry Sci. 54:217-221.
7. Rebers, P.A., K.L. Heddleston, B. Wright, and K. Gillette, 1975. Fowl cholera: Cross-protective turkey antisera and IgG antibodies induced with Pasteurella multocida infected tissue bacterins. Carbohydrate Res. 40:99-110.
8. Rebers, P.A. and K.L. Heddleston, 1977. Fowl cholera: Induction of cross-protection in turkeys with bacterins prepared from host-passged Pasteurella multocida. Avian Dis. 21:51-56.
9. Rimler, R.B., P.A. Rebers, and K.R. Rhoades, 1979. Fowl cholera: Cross-protection induced by Pasteurella multocida separated from infected turkey blood. Avian Dis. 23:730-741.
10. Rimler, R.B., P.A. Rebers, and K.R. Rhoades, 1980. Modulation of cross-protection factor(s) of avian Pasteurella multocida. Avian Dis. 24:989-998.

THE DEVELOPMENT OF A MONOCLONAL ANTIBODY FOR THE DETECTION AND DIFFERENTIATION OF THE AVIRULENT VACCINE STRAIN FROM THE VIRULENT STRAIN OF HEMORRHAGIC ENTERITIS VIRUS (HEV)

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INTRODUCTION

Hemorrhagic enteritis is an acute infectious viral disease of four week or older turkeys. Characteristic signs of the disease are depression, bloody droppings and sudden death. The disease is caused by a type II avien adenovirus that is indistinguishable from that causing marble spleen disease in pheasants and that causing splenomegaly in chickens.[1]

The disease has been reported from all major turkey raising areas of the United States. Hemorrhagic enteritis has been reported in both range and confinement reared turkeys. The disease has a strong tendency to infect successive flocks on a premise.[2]

Natural transmission of hemorrhagic enteritis is by the oral route. In the acute form of the disease the most common clinical signs involve the intestines, which are distended and filled with blood. Spleens are usually enlarged and marbled or mottled in appearance.[3,4]

A monoclonal antibody is being developed from the hybridization of murine myeloma cells and splenocytes from mice immunized with purified HEV.[5,6] Presently, the avirulent strain (pheasant origin) is being extensively used to vaccinate turkeys against H.E. Currently there is no method available to distinguish these two viruses serologically.

A monoclonal antibody can be a valuable tool for identifying and differentiating the vaccine strain (pheasant origin) from the virulent strain (turkey origin) of the virus.

Differentiation of the virulent strain from the vaccine strain of HEV is imperative in order to initiate appropriate control measures for the wide use of the cell culture propagated vaccine virus.

METHODS

Both the vaccine and virulent viruses were propagated in cell culture and purified in Cesium chloride (CsCl).[7] The purified virus was used for the hyperimmunization of Balb/c mice and as a viral antigen for use in the Enzyme Linked Immunosorbent Assay (ELISA). ELISA was employed for the screening of hybridoma supernatants.

Mouse Immunization

Six-to-eight week old female Balb/c mice were immunized by priming intraperitoneally (IP) with the appropriate virus. Two further IP boosts of

antigen were administered over the following 2 to 3 months. A final intravenous (IV) booster of antigen was given 3 to 4 days before the mice were sacrificed, at which time the spleens were removed aseptically.[8]

Production of hybridomas

Spleens from immunized animals were disrupted to release individual splenocytes. The cell suspension was washed in serum free Dulbecco's minimal essential medium (DMEM) and resuspended in DMEM at a concentration of 10^7 cells/ml. Meanwhile the myeloma cells which had been growing in the exponential phase were washed and also resuspended at a concentration of 10^7 cells/ml.[8]

The hybridization method was basically quite simple, namely, the spleen cells from the hyperimmunized mice were mixed with the myeloma cells. The mixture of cells was then incubated for a short time in the presence of 50% polyethylene glycol. Following this incubation, aliquots of the cell mixture were cultured under conditions that allow only hybrid cells to survive.

Once the hybrid cells are growing, the cultures are tested for the production of desired antibody using ELISA.

RESULTS AND DISCUSSION

A monoclonal antibody can be used to identify and differentiate the vaccine strain from the virulent strain of H.E.V. If a unique antigenic determinant is expressed exclusively on either the virulent or vaccine strain, then a monoclonal antibody might be identified that could be used to identify and differentiate these two viruses.

At the present time over 3,000 hybrid clones have been screened for the production of desired antibody. Of these 3,000 clones tested, over 300 have produced specific antibody against H.E. antigen. However, the antibody produced has cross reacted with both the virulent and vaccine strain of the viruses and as of yet no antibody has been isolated that is specific to one strain of the H.E. virus. Further, screening the remaining clones is in progress in an attempt to identify the clone that is producing specific antibody that does not cross react between the vaccine and virulent strains.

LITERATURE CITED

1. Domermuth, C.H. and J.R. Harris, 1974. A naturally occurring infection of chickens with a hemorrhagic enteritis/marble spleen disease type of virus. Avian Diseases, 23:479-484.
2. Thorsen, J. and L. Weninger, 1981. Field trials of an immunization procedure against hemorrhagic enteritis of turkeys. Avian Diseases, 26:473-477.
3. Gross, W.B. and C.H. Domermuth, 1975. Spleen lesions of hemorrhagic enteritis of turkeys. Avian Diseases, 20:455-466.
4. Sponenberg, D.P. and C.H. Domermuth, 1985. Field outbreaks of colibacillosis of turkeys associated with hemorrhagic enteritis virus. Avian Diseases, 29:838-842.
5. Christopher, L.R, 1982. Theory and methods of immunization in culture and monoclonal antibody production. J. Immun. Methods, 53:251-291.

6. Kohler, G., 1981. The technique of hybridoma production. J. Immun. Methods, 11:285-298.
7. Fadly, A.M. and K.V. Nagaraja, 1985. Field vaccination against hemorrhagic enteritis of turkeys by a cell-culture live-virus vaccine. Avian Diseases, 29:768-777.
8. Russell, W.C. and Gunvanti, P., 1981. Monoclonal antibodies against adenovirus type 5: Preparation and preliminary characterization. J. Gen. Virol., 56:393-408.

AN ENVIRONMENTAL MONITORING SYSTEM FOR AVIAN INFLUENZA

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INTRODUCTION

As an epidemiological tool, monitoring the environment for the presence of avian influenza helps us to define the relationship between the presence of influenza in nature and outbreaks on poultry farms. Knowledge of the specific means of interspecies transmission between waterfowl(the primary reservoir) and poultry flocks might allow the development of better methods for preventing these influenza outbreaks.

At the present time, monitoring waterfowl for the presence of influenza requires cumbersome methods for capturing wild birds, or the use of sentinel ducks, which are birds placed in cages on ponds and periodically tested for influenza(Halvorson et al, 1985). A simpler and more sensitive procedure is needed in order to routinely monitor the environment for the presence of influenza. The ability to concentrate and detect influenza in the water in which the waterfowl reside would be a useful monitoring tool. This virus concentration and detection method would be applicable to both surface and groundwater studies for possible waterborne transmission of influenza(Hinshaw et al, 1979; Shortridge et al, 1982).

The fecal deposition of virus particles by waterfowl into surface pond water results in dilution, so that the number of virus particles in a given volume is expected to be quite small. Therefore, a concentration procedure which allows efficient recovery of virus with reduction in volume to an amount which is easy to assay is highly desireable. The viradel (virus adsorption-elution) method is a procedure which has been used in the detection of human enteroviruses in large volumes of surface water and wastewater(Farrah et al, 1976). The procedure involves using microporous filters through which virus-contaminated water is passed, allowing adsorption of viruses to the filters due to electrostatic and hydrophobic interactions, rather than physical entrapment of virus particles(the filter pore size is larger than the diameter of the virus particle). Small volumes of alkaline buffers are then used to release (elute) the virus particles from the filter, resulting in a concentrated solution containing the virus. The virus can then be concentrated even further using various techniques better suited for small volumes, and identified through the use of an appropriate system such as cell culture or egg inoculation.

MATERIALS AND METHODS

Influenza A/Duck/Mn/613/79 (H4N2) was used as the model virus for developing the viradel procedure for influenza. A standard hemagglutination test was used in order to quantitate the virus before and after subjecting it to the viradel concentration technique. Influent(the solution to be filtered), effluent(solution that has passed through the filter), and eluate(fluid containing the concentrated virus) were titrated in duplicate, and averaged where appropriate. Initial and final volumes were also noted, permitting the calculation of percent virus adsorption, percent virus recovery, and an overall virus concentration factor for the procedure.

$$\text{Percent adsorption} = \frac{\text{influent titer} - \text{effluent titer}}{\text{influent titer}} \times 100\%$$

$$\text{Percent virus recovery} = \frac{\text{eluate titer} \times \text{eluate volume}}{\text{influent titer} \times \text{influent volume}} \times 100\%$$

Overall virus concentration factor = # times water volume reduced x % virus recovery

Twenty-five millimeter membrane filter holders(Sartorius, Gottingen, Fed. Rep. Germany) were used in the small volume experiments. Virosorb 1-MDS filter material was supplied by Cuno Division, Meriden, Conn. Filters came in sheets that could be cut to fit the flat disk filter holders, or in pleated cartridge form(for use on large water volumes) with cartridge filter holders.

Small water volume studies

Influent pH and Adsorption. A virus stock solution was prepared by adding virus to distilled water and adjusting to pH 9.8. A portion of this solution (influent) was then passed dropwise through a filter holder and the effluent collected and titrated. Ø.Ø5M Glycine pH 11.5 was then passed through the filter to cause virus release. The pH of the virus stock solution was reduced to various levels and the filtration/titration process repeated each time.

Flow Rate and Adsorption. Influent was passed through each of three filters at flow rates of 32, 48, and 96 ml/minute. Percent virus adsorption and recovery were calculated as shown above.

Eluent pH and virus recovery. An equal volume of influent was passed through each filter. Ø.Ø5M Glycine(the eluent) was adjusted to six different pH values, and used to cause virus release from each filter. Percent virus recoveries were compared to determine the ideal eluent pH for maximum recovery of influenza virus.

Eluent content and virus recovery. An equal volume of influent was passed through each of 1Ø filters. The filters were

then eluted with various solutions designed to cause virus release from the filter. Percent virus recovery was compared to determine the best eluent for influenza virus.

Large water volume studies

Comparison to small volume model. 100 liters of dechlorinated tap water was adjusted the desired pH and seeded with influenza virus. The water was passed through a pleated cartridge filter using an electric pump, and 0.05M Glycine pH 11.5 was used to elute the virus from the filter. This eluate was then titrated for HA activity, its volume noted, and percent virus recovery calculated.

Second-step concentration methods. Eluates generated in the large water volume studies were subjected to two different techniques to further concentrate the virus, and therefore increase the sensitivity of our detection system. In the first experiment, a method for concentrating influenza virus from allantoic fluid(Heyward et al, 1977) was modified for use on the glycine eluate. The eluate pH was reduced to 8.0 and titrated. Polyethylene glycol 20,000 MW(PEG) was added to the eluate (8% w/v) and kept at 4C for one hour. The eluate was then centrifuged at 10,000 x g for 20 min at 4C. The supernatant was discarded and the remaining pellet was resuspended in a small volume of a buffered saline solution. A percent virus recovery was then calculated to evaluate the effectiveness of the procedure.

The second technique tested involved precipitation of influenza virus with saturated ammonium sulfate(Shields et al, 1986). Two volumes of saturated ammonium sulfate were added to one volume of eluate and immediately centrifuged at 14,500 x g for 15 minutes. The supernatant was then discarded and the pellet resuspended in buffered saline. Percent virus recovery was calculated.

RESULTS

Influent pH

(see table 1) 75-100% of the virus particles adsorbed to the 1-MDS filter over the pH range 6.6-8.3. When adsorption and recovery data are considered together, pH 7.3 appears to result in the greatest virus adsorption. Even at pH 9.3, 50% adsorption occurs, however,virus recovery rates at this pH value are low. Below pH 6.6, a decline in HA titer is seen, which is probably due to the acid-sensitive nature of influenza(Scholtissek, 1985). Reliable absorption and recovery data at these values is therefore difficult.

Flow rate

(see table 2) Influent pH 7.3 was used when testing different flow rates based on previous data showing this pH as producing maximum virus(H4N2) adsorption to the filter. In this way, flow rate could be evaluated without introducing influent pH as

another variable affecting virus adsorption. Flow rates up to 96 ml/min resulted in 100% adsorption. When filter surface area is taken in account, this means that up to 5 gallons of water per minute can be processsed using cartridge-type filters. Influent pH 9.8 was tested in order to control for possible virus particle damage and loss of hemagglutinating activity due to forces that might be generated at high flow rates. At influent pH 9.8, virtually no virus adsorption would be expected, therefore, if no virus damage occurred at high flow rates, influent and effluent HA titers at this pH should be similar, which proved to be the case in this experiment.

Eluent pH

(see table 3) Eluent pH appears to be very important in causing release of the virus from the filter. As pH increases, percent virus recovery increases. Eluent pH 11.5 resulted in the best virus recovery rate.

Eluent content

(see table 4) 0.05M Glycine and 0.05M Glycine + 0.1M NaCl(pH 11.5) achieved the best virus recovery rates. High concentrations of NaCl in the eluent seemed to result in the lowest virus recovery rates.

Large water volumes using cartridge filters

(see table 5) Percent virus recovery ranged from 39% at influent pH 7.3 to 54% at pH 7.5. In one trial at pH 7.5, the viradel procedure effectively concentrated the virus 31 times.

Second-step concentration

(see tables 6 and 7) The most effective means of concentrating the virus using the "PEG" method seemed to involve reducing the eluate volume by a factor of 10, which effectively concentrated the virus 12-fold. The ammonium sulfate method concentrated the virus 10-fold when the eluate volume was decreased by a factor of 21.

DISCUSSION AND SUMMARY

The following conclusions can be drawn from this study:

1) Since the influent pH range over which influenza virus will adsorb to the filter is quite broad, little or no pH alteration of the water is necessary prior to filtration. This simplifies the viradel procedure when large volumes of water are filtered.
2) Flow rates of 5 gal/min(possibly higher) will not interfere with adsorption of virus to the filter. Therefore, the filtration of large water volumes can be performed rapidly.

3) The best eluent for the recovery of influenza virus appears to be Ø.Ø5M Glycine pH 11.5.
4) The results of the large water volume studies compare favorably with the small volume model.
5) The viradel technique, in combination with the "PEG" second-step method can effectively concentrate influenza virus up to 372 times when performed on 1ØØ liters of tap water. Tests comparing lake water and distilled water showed that virus could be concentrated more effectively in lake water(data not shown), therefore, virus concentration in field studies on surface water may be even higher than the results achieved in the laboratory.

LITERATURE CITED

Farrah, S.R., C.P. Gerba, C. Wallis, and J.L. Melnick. 1976. Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Environ. Microbiol. 31:221-226.

Halvorson, D.A., C.J. Kelleher, and D.A. Senne. 1985. Epizootiology of avian influenza: Effect of season on incidence in sentinel ducks and domestic turkeys in Minnesota. Appl. Environ. Microbiol.49:914-919.

Heyward, J.T., R.A. Klimas, M.D. Stapp, and J.F. Obijeski. 1977. The rapid concentration and purification of influenza virus from allantoic fluid. Arch. Virol. 55:1Ø7-119.

Hinshaw, V.S., R.G. Webster, and B. Turner. 1979. Waterborne transmission of influenza A virus? Intervirology 2:66-68.

Markwell, D.D., and K.G. Shortridge. 1982. Possible waterborne transimission and maintenance of influenza viruses in domestic ducks. Appl. Environ. Micro. 43:11Ø-116.

Scholtissek, C. 1985. Stability of infectious influenza A viruses to treatment at low pH and heating. Arch. Virol. 85:1-11.

Shields, P.A., and S.R. Farrah. 1986. Concentration of viruses in beef extract by flocculation with ammonium sulfate. Appl. Environ. Microbiol.51:211-213.

Table 1. Effect of influent pH on virus adsorption[a]

Influent pH	% virus adsorption	% virus recovery (elution)
5.3	100	25
5.9	100	25
6.2	100	21
6.6	100	0
7.0	75	50
7.1	100	56
7.3	100	88
7.4	100	56
7.7	88	38
7.9	75	25
8.0	75	25
8.3	75	50
8.6	63	29
9.1	50	9
9.3	50	9
9.8	0	9

a 16ml distilled water containing virus (HA titer= 8) was passed through a filter at different pH values. Each filter was then eluted with 4ml 0.05M Glycine pH 11.5. Percent adsorption and recovery were calculated as described in materials and methods.

Table 2. Effect of flow rate on virus adsorption[a]

Flow rate (ml/min)	influent pH	HA titer			%ads	%rec
		influent	effluent	eluate		
32	7.3	8	0	15	100	46
	9.8	8	8	1	0	3
48	7.3	8	0	12	100	38
	9.8	8	8	2	0	6
96	7.3	8	0	12	100	38
	9.8	8	8	0.5	0	1

a 16ml virus in distilled water was passed through a filter at various flow rates. 0.05M Glycine pH 11.5 was then used to elute each filter. Each result represents the average of 2-3 trials.

Table 3. Effect of eluent pH on virus recovery from filters[a]

Eluent pH	% virus recovery
9.Ø	Ø
9.5	Ø
1Ø.Ø	Ø
1Ø.5	8
11.Ø	23
11.5	33

a 8ml virus in distilled water(HA titer= 8) adjusted to pH 7.1 was passed through a 25mm diameter Virosorb 1-MDS filter. 4ml Ø.Ø5M Glycine (adjusted to various pH values) was used to elute the virus. All effluent titers were zero. The results are the average of three trials conducted at each pH value.

Table 4. Effect of different eluents on virus recovery[a]

Eluent[b]	Percent virus recovery distilled water	tap water
Ø.Ø5M Glycine(Gly)	67	59
Ø.Ø5M Gly + Ø.1M NaCl	67	63
Ø.Ø5M Gly + Ø.5M NaCl	33	38
3% Beef extract(BE)	33	38
Ø.Ø5M Gly + 3% BE	42	27
1M NaCl	29	29
Ø.Ø5M Gly + 1M NaCl	29	23
Ø.Ø5M Gly + 1M NaCl + Ø.5% BE	13	32
Ø.Ø5M Gly + 1M NaCl + 3% BE	21	19
3% BE + 1M NaCl	17	2Ø

a 3Ømls H4N2 in distilled or tap water was adjusted to pH 7.1-7.8 (HA titers of the influent ranged from 8-96) and passed through a 25mm diameter Virosorb 1-MDS filter. All effluent titers were zero. The filter was then eluted with 2ml eluent. The results represent the average of two trials.

b All eluents were adjusted to pH 11.5

Table 5. Pleated cartridge filter performance using 100 liters of dechlorinate tapwater[a]

pH	eluate volume(L)	volume reduced by a factor of:	% virus recovery	virus concentrated by a factor of:
7.3	1.85	54	39	16
7.5	1.77	56	42	24
7.5	1.73	58	54	31
8.2	1.66	60	50	30

a 100 liters of tap water seeded with a known volume and HA titer of influenza virus(H4N2)was filtered through a cartridge filter, eluted with ~ 1.6 liters 0.05M Glycine pH 11.5, and the eluate volume and HA titer determined.

Table 6. PEG 20,000 MW concentration method[a]

final volume(ml)	volume reduced by a factor of:	% virus recovery	virus concentrated by a factor of:
48	8	89	7
23	16	72	12
13	30	73	2
7	53	6	3
3	127	6	8

a 350-400ml 0.05M Glycine eluate containing H4N2 virus was neutralized to pH 8.0 with 1N HCl and titrated. PEG 20,000 MW(8% w/v) was added and incubated at 4C for one hour. The solution was then centrifuged at 10,000 x g for 20 min. @ 4C. The supernatant was discarded and the pellet resuspended in a buffered saline solution, its volume noted, and titrated. The results represent an average of 1-5 trials.

Table 7. Saturated ammonium sulfate method[a]

final volume (ml)	volume reduced by a factor of:	% virus recovery	virus concentrated by a factor of:
8	8	34	3
4	16	29	5
3	21	47	10
2	32	24	8

a 64ml 0.05M Glycine eluate was titrated for HA activity. Two volumes of saturated ammonium sulfate was added, and the mixture centrifuged at 14,500 x g for 15 min. The supernatant was discarded and the pellet resuspended in a buffered saline solution. The volume was again noted and the solution titrated for HA activity. The results represent the average of 2-8 trials.

ARTIFICIAL INSEMINATION TECHNIQUES

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Basic concepts regarding semen quality and sperm-oviduct interrelationships will be presented. Semen quality, semen quality tests and oviductal sperm transport will be discussed, as will sperm storage within the oviduct. The effects of A.I. techniques on oviductal sperm storage and fertility will also be addressed. A review of the concepts regarding sperm-oviduct interrelationships should aid in placing current artificial insemination practices into perspective, and should allow distinguishing between desirable and undesirable insemination practices.

ASPECTS OF SEASONAL DECLINE IN FERTILITY

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So-called seasonal declines in fertility are a persistant and prominent fertility problem observed in nearly all commercial turkey breeder flocks. Seasonal declines in fertility are characterized by a gradual drop in fertility over the course of an egg-laying period or season, ultimately declining 20 to 25% after approximately 20 wk of egg production. The decline in fertility occurs despite an increased frequency of insemination (Nestor and Brown, 1968; Meyer, et al., 1980; Christensen, 1981; McIntyre and Christensen, 1985) or an increased number of spermatozoa inseminated (Meyer et al., 1980; Ogasawara and Rooney, 1966; Sexton, 1977). Because fertility remains poor in spite of repeated inseminations with semen of proven quality, the problem appears to be female-related.

Immunological effects have been investigated as a cause of seasonal declines in fertility. Burke et al. (1971) reported that turkey hens isoimmunized with semen had lower fertility than nonimmunized hens. Burke and Reiser (1972) found that when spermatozoa were incubated in blood serum from infertile hens prior to insemination, significantly lower levels of fertility were obtained. However, antisperm antibodies were not detected when standard immunological tests were used. Yu and Burke (1979) showed that gamma globulins from turkey hens immunized against semen also depressed the fertilizing capacity of incubated spermatozoa. More recently, McCorkle et al. (1983) adapted the Friberg (1974) microagglutination test to demonstrate the presence of antisperm antibodies in the blood serum of artifically inseminated turkey breeder hens.

Ball et al. (1969) were not able to associate any of the more common pathogens with turkey infertility. They did, however, note increased numbers of lymphoid foci in the lamina propria of the infundibular and isthmal regions of the oviduct and also noted an infiltration of plasma cells in association with the lymphoid foci. Recently, we reported finding plasma cells within the primary oviductal sperm storage site, the uterovaginal sperm storage glands, of infertile turkey breeder hens experiencing a seasonal decline in fertility (Van Krey et al., 1987).

Uterovaginal sperm storage glands taken from fertile and infertile turkey breeder hens were analyzed morphologically using transmission electron microscopy. Sperm storage glands from the infertile hens were generally devoid of spermatozoa, while glands from the fertile hens contained many such cells. Extensive lymphocytic infiltration into the basolateral clefts between contiguous cells of the sperm glands were found in both fertile and infertile hens. Plasma cell infiltration into these intracellular clefts was also found in infertile turkeys. Plasma cells were not found, however, in the glandular clefts of fertile hens.

Lymphocytes present in the sperm storage glands of fertile hens are theorized to be regulatory suppressor T-cells, which could explain the hen's immunological tolerance to continual exposure to antigenic spermatozoa. Conversely, the presence of antibody-producing plasma cells in the sperm storage glands of infertile hens could explain the absence of stored spermatozoa and the reduced fecundity of these hens.

REFERENCES

Ball, R. A., V. B. Singh and B. S. Pomeroy, 1969. The morphological response of the turkey oviduct to certain pathogenic agents. Avian Dis. 13:119-133.

Burke, W. H. and J. W. Rieser, 1972. The effects of antisperm antisera and uterine fluids on the fertilizing ability of turkey spermatozoa. Poultry Sci. 51:190-195.

Burke, W. H., J. W. Rieser and R. N. Shoffner, 1971. The effects of isoimmunization with semen on fertility in the turkey hen. Poultry Sci. 50:1841-1847.

Christensen, V. L., 1981. Effect of insemination intervals on oviductal sperm storage in turkeys. Poultry Sci. 60:2150-2156.

Friberg, J., 1974. A simple and sensitive micro-method for demonstration of sperm-aggultinating antibodies in serum from infertile men and women. Acta Obstet. Gynecol. Scand. Suppl. 36:21-29.

McCorkle, F. M., V. L. Christensen and J. P. Thaxton, 1983. Anti-sperm antibodies and fertility of turkey hens. J. Reprod. Immunol. 5:363-370.

McIntyre, D. R. and V. L. Christensen, 1985. Effect of initial insemination and insemination interval on fertility in turkey hens. Poultry Sci. 64:1549-1552.

Meyer, G. B., C. F. Props, A. T. Leighton Jr., H. P. Van Krey, and L. M. Potter, 1980. Influence of dietary protein during the pre-breeder period on subsequent reproductive performance of Large White turkeys. 3. The effects of semen volume and frequency of insemination on fertility and hatchability. Poultry Sci. 59:363-368.

Nestor, K. E. and K. I. Brown, 1968. Method and frequency of artificial insemination and turkey fertility. Poultry Sci. 47:717-721.

Ogasawara, F. X. and W. F. Rooney, 1966. Artificial insemination and fertility in turkeys. Br. Poultry Sci. 7:77-82.

Sexton, T. J., 1977. Relationship between number of sperm inseminated and fertility of turkey hens at various stages of production. Poultry Sci. 56:104-110.

Van Krey, H. P., G. T. Schuppin, D. M. Denbow, and R. M. Hulet, 1987. Turkey breeder hen infertility associated with plasma cells in the uterovaginal sperm storage glands. Theriogenology 27:913-921.

Yu, W. C. Y. and W. H. Burke, 1979. Infertility in the turkey. III. Effects of sera from fertile and infertile females on the fertilizing ability of spermatozoa. Poultry Sci. 58:1378-1381.

A PHILOSOPHICAL APPROACH TO SEMEN PRESERVATION

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INTRODUCTION

Since 1968 when scientists of the University of Minnesota and the Minnesota Turkey Growers Association first made a turkey semen extender commercially available, many changes in the industry have occurred. This advent allowed the turkey breeder to reduce the number of male birds needed with considerable cost savings. Improved methods of semen preservation has also given some flexibility between semen collection and time of insemination without loss in fertility. Having this flexibility also allows the breeder opportunity for critical semen evaluation. With this advantage the breeder can more closely monitor and adjust proper cell numbers for insemination and eliminate poor quality semen. As semen preservation techniques are improved and longer term storage of spermatozoa is becoming feasable more breeders are establishing tom housing facilities and preparing for stud farms. When this is achieved, greater selection pressures can be exerted, higher quality semen obtained, and semen can be shipped greater distances for use.

It is the author's opinion that within a relatively short time 24-48 stored liquid semen will be available that yields normal or higher fertility than is obtained at present. The primary objective of this paper is to briefly describe a few criteria necessary to achieve use of longer term stored spermatozoa.

INITIAL SEMEN QUALITY

Requisite to longer term storage of semen, with consistant results, is high quality semen. Two possible approaches include: (1) pre-selection of toms and/or (2) elimination of poor quality semen after collection. Selection of toms for semen quality just prior to or at the commencement of insemination assures the flock owner that high quality semen is available and allows quick culling of non-productive birds. Tables 1 and 2 show the percentages of birds that may need culling under two different methods that may be used to evaluate semen quality. Previous reports (6)(16), indicate that by use of semen evaluation assay and inseminating semen to groups of birds based on the assay the percentage fertility is reflected by semen so classified.

Table 1. Percentage of toms yielding a swirling action after 6, 12, 24, 36 hrs storage*.

	Storage time				
# Toms	6 hrs	12 hrs	24 hrs	36 hrs	r^{xx}
820	72	64	52	14	.84

*5°C rotated 10 RPH.

Table 2. The use of an index* for semen quality for selecting high, medium and low.

	Index				
# Toms	<50	50-100	100-150	150+	r^{xx}
248	19	22	37	26	.76

*Volume x sperm/ml x % motility.

The data in Table 1 classifies semen into four groups using only longevity of spermatozoa stored under controlled conditions. Semen from approximately 28% of a flock can not maintain its quality for as little as 6 hours, 64% for 12 hours, 52% for 24 hours and only 14% for up to 36 hours. Statistically when the birds are analyzed with this assay the same birds will show similar quality 84% of the time. Using a more rigorous assay combining 3 criteria for semen quality fewer birds would be classified as superior in quality with repeatability at 76%. Using an index accounting for volume of semen, spermatozoa concentration and motility at 6 hours, 19% of the birds would be culled, 26% considered superior, 37% good and 22% fair. These type data are not definitive since seldom are individual sires used for insemination. They may be useful in determining numbers of males necessary to achieve a longer term storage program.

Once toms have been preselected and divided into smaller groups, the individual groups could be monitored for semen quality. Likewise if individual birds were collected, these samples could be assayed and the good samples pooled for insemination.

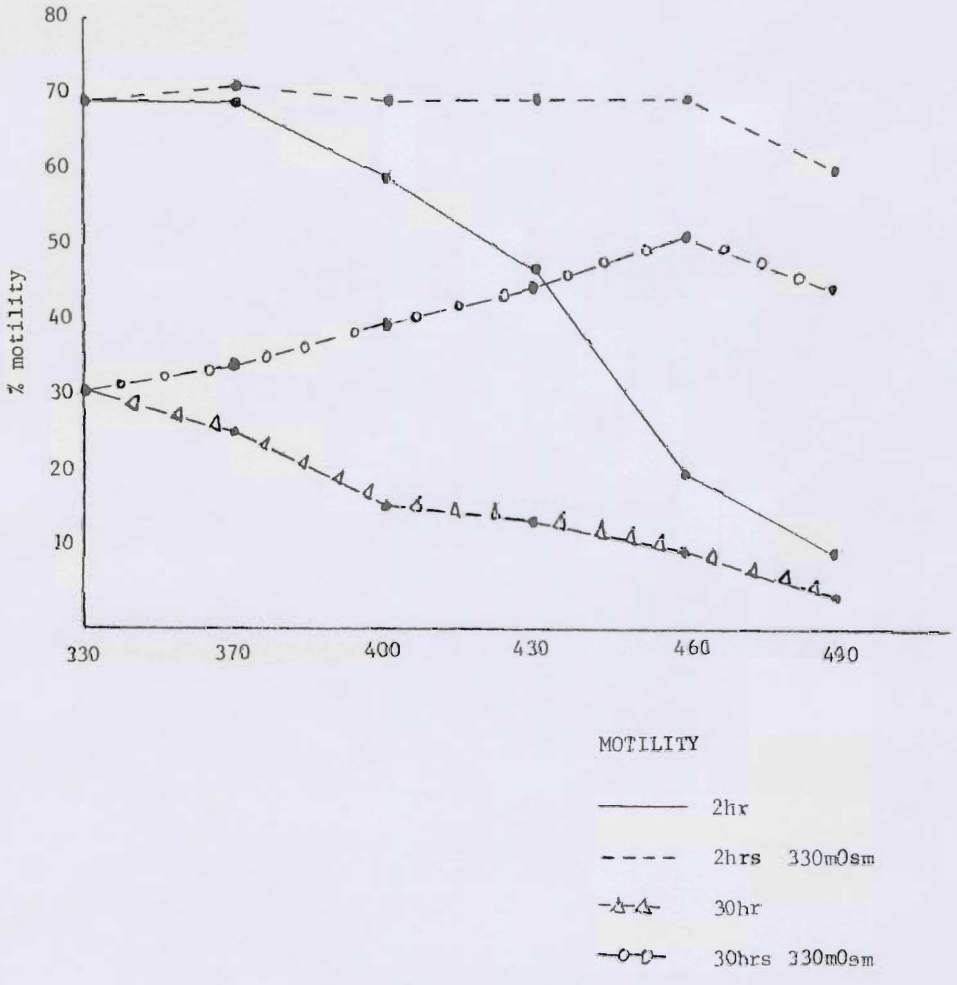
SEMEN EXTENDERS

An absolute requirement for longer term preservation of turkey spermatozoa is an appropriate buffer or extender. Several acceptable extenders have been reported (1)(8)(13) as being effective and several are commercially available to the turkey breeder. Attempts have been made to conduct comparative effects on fertility (1)(7)(9). Variable results have been reported but longer term storage of semen generally results in decreased fertility as compared to the control. Possible exceptions include reports of Wambeke (16) and it appears that most extenders described allow storage of spermatozoa for at least 6 hrs without loss of fertility. From the literature it is difficult to try to assess the qualities of each type of extender and to draw a conclusion as to the specific ingredients that make them effective. In general all contain one or several buffering systems which are essential to a good extender. Several contain zwitter ionic buffers (Good's buffers) that are effective with a pK in the biological range appropriate to spermatozoa, and a pH of 6.5-7.5 (3)(11). Some employ phosphates which exerts a buffering capacity in this pH range (9). Little consensus is found in reports to define the proper pH of an extender for longer term storage. The possible reason is that there is an apparent interaction between pH and specific ingredients, temperature of storage and methods of handling (9)(13).

Likewise, equal results are often reported by different researchers using extenders of widely different pH. It is apparent that several different extenders are effective over the pH range of 6.3-7.5. Metabolism of spermatozoa has been shown to decrease as pH is lowered prolonging the livability of the cell. Further research in this area is indicated.

Another requirement of an extender is an osmotic pressure which is isosmotic to the cell. Most extenders used have sufficient dissolved materials to maintain an osmotic pressure of 330-460 mOsm/kg (1)(13)(16). It has been difficult to test the differences in fertility due to osmotic pressure because to alter osmotic pressure different amounts of dissolved materials or different materials are used which alter the chemical composition and/or the ionic strength of the solution. In reviewing the recent literature it appears that the most effective extenders have an increased osmolarity (1)(16). It is known that freshly collected seminal plasma contains an osmotic pressure of about 315 mOsm/kg and that vas deferentia plasma, where cells are stored for long periods of time, has 440 mOsm/kg. Increased osmotic pressure decreases motility of spermatozoa but increases their livability (Fig. 1). Likewise, cells placed in hypertonic solutions decrease the number of "neck bent" cells.

Figure 1. The effect of extender osmotic pressure on motility of turkey semen extended 1:3 before and after readjustment to 330 mOsm/kg stored for 2 or 30 hours.



Although several claims have been made of the achievement of high fertility after 24-48 hour storage in small controlled studies, there is little evidence that it can be achieved on a routine flock basis. Further work on extenders is suggested. To refine specific extender ingredients it may be advisable to re-evaluate differences in seminal plasma as collected and the chemical composition of vas deferentia fluid (Table 3). The data shown suggest extenders should contain at least a higher pH, a high osmotic pressure (440 after final dilution), a predominance of phosphorus, glutamate, serine, a decrease in chloride and a Na:K ratio of 1:1.

Table 3. A comparison between some physical, physico-chemical and chemical components of turkey seminal plasma and vas deferens fluid.

	Seminal Plasma	Vas Fluid
Spermatozoa/ml x 10^9	9.4	22.7
pH	7.2	7.8
Osmotic pressure, mOsm/kg	305	440
Sodium MEQ/L	166.6	52.0
Potassium MEQ/L	16.8	48.6
Chloride MEQ/L	30.7	18.2
Phosphorus, mg%	4.1	12.2
Glutamic acid, µM/ml	86.5	152.2
Serine acid, µM/ml	6.5	16.7

DILUTION RATIO

For an extender to be most effective for semen storage there is need to increase the ratio of extender to semen. Tables 4 and 5 illustrate this phenomenon. Others have suggested ratios of 1:4 and 1:5 are also effective. The caution of high extension ratios is that sperm numbers per insemination must be taken into consideration. The proper number of cells per insemination has been reviewed by many investigators so will not be covered here.

SEMEN HANDLING AFTER EXTENSION

For longer term semen storage two or three important factors must be considered. These include temperature of storage, aeration and/or agitation. In recent years data indicate that storage temperatures of 3-7°C is superior to higher temperatures (1)(17). At lower temperatures there is a decrease in cell metabolism and livability is prolongated.

For many years it has been known that turkey spermatozoa require oxygen if longer term storage is desired. Many methods of oxygenation have been suggested

Table 4. Effect of dilution ratio and motility of buffered semen (selected males) under three different storage conditions.

Dilution Ratio	0 hr	6 hr	12 hr	18 hr	24 hr	36 hr	48 hr
			---- Storage 5°C ----				
1:1	S*	55	45	40	20	0	0
1:2	S	S	55	50	30	10	0
1:3	S	S	S	60	50	40	5
			---- Dialyzed (Ratio 1:10) ----				
1:1	S	S	S	60	50	-20	0
1:2	S	S	S	S	S	55	10
1:3	S	S	S	S	S	S	50
			----Under appropriate O_2 (No dialysis) ----				
1:1	S	S	55	30	10	5	0
1:2	S	S	S	S	60	40	0
1:3	S	S	S	S	S	S	55

*S - Swirling motility
Significant difference between dilution ratios on time.
Significant difference between no treatment and treatments.
No significant difference between dialysis and O_2.
Possible storage for 36 hours at 1:3 dilution.

Table 5. Comparison of 2 types of storage methods and dilution ratio of fertility of turkey spermatozoa.

	No. Eggs	No. Fert.	% Fert.
	---- Dialysis ----		
1:1	294	(269)	88
1:3	312	(284)	91
	---- O_2 Method ----		
1:1	310	(269)	87
1:3	305	(276)	90

Selected toms; Six birds per treatment; 14 week lay; Insemination 7 day intervals.

Equal sperm numbers / insemination
Dosage approzimately 100 x 10^6

6-8 hour storage + 5°C

and all are more effective than static storage. Methods of aeration include agitation (1)(11), continuous or intermittent (10), bubbling oxygen into the sample (19) and the use of oxygenated flourocarbons (4). The former methods require vigorous agitation that could be damaging to the cell. Our work also indicates the necessity of aeration (Table 6). Our method of aeration is simple yet effective. Extended semen is placed on a slanted wheel in an environment of 5°C. The semen quantity and vessel size is proportional to yield a 1:3 volume to surface ratio. The wheel is turned at 10 RPH. The vial is in a fixed position on wheel resulting in a smooth rolling action. The vessel is left unstoppered during the storage period. The oxygen content of the turned sample is approximately 220-240 nmoles/ml as contrast to 50-60 nmoles/ml when held in the still state.

Table 6. Comparison of 6 hour and 30 hour stored turkey semen stored at 5°C static and agitated.

	6 hr storage			30 hr storage			Total		
Treatment	# Eggs	# Fert.	% Fert.	# Eggs	# Fert.	% Fert.	# Eggs	# Fert.	% Fert.
Static	2560	2227	86.9[b]	2610	2036	78.0[b]	5180	4263	82.3[b]
Agitated*	2480	2207	89.9[a]	2570	2108	82.0[a]	5050	4315	85.4[a]
Total	5040	4434	87.9[a]	5180	4144	80.0[b]			

Agitated*--turned at 5 RPH for 6 hr prior to insemination.
Once/wk insemination; 20 weeks; 100 x 10^6 sperm/insemination; 1:3 semen to extender.
[a,b]Different superscripts in the same vertical line are significant.

CONCLUSIONS

Great progress has been made in efforts to achieve longer term preservation of turkey spermatozoa. Chemically defined extenders are continually being improved. The importance of initial semen quality is emerging with many flock owners putting in equipment to monitor quality and sperm numbers. Greater understanding of dilution effect and methods of semen handling are emerging. Lastly, the flock owners are beginning to trust their ability to store semen for longer periods of time resulting in higher known quality of semen throughout the breeding season.

REFERENCES

1. Cherms, F.L., 1981. Effect of diluent and agitation on short term storage of turkey semen. Poult. Sci., 60(7): 1638-9, abstr.

2. Clarke, R.N., M.A. Ottinger and T.J. Sexton, 1981. The effect of temperature and storage time on the respiration, motility and fertility of chicken and turkey semen. Poult. Sci., 60(7): 1640, abstr
3. Clarke, R.N., T.J. Sexton and M.A. Ottinger, 1982. Effects of holding temperature and storage time on respiratory rate, motility, and fertility of chicken and turkey semen. Poult. Sci., 61: 1912-1917.
4. Froman, D.P., 1982. Factors potentially affecting the fertilizing ability of in vitro stored turkey spermatozoa. Diss. Abstr. Int'l., 43: 639B.
5. Giesen, A.F., 1983. Preservation of turkey semen at temperatures above 0°C. Diss Abstr. Int'l., 43: 3149B-3150B.
6. Graham, E.F., 1985. Semen quality and its preservation.
7. Huyghebaert, G. and G. de Groote, 1983a. The effect of diluent, storage temperature and number of spermatozoa on fertility and hatchability results obtained with turkey semen stored for 6 hours. Arch. Geflugelk., 47: 103-109.
8. Huyghebaert, G., F. van Wambeke and G. de Groote, 1983b. The effect of diluent, dilution rate and number of spermatozoa on fertility and hatchability obtained with turkey semen stored for 2 hours. Arch. Geflugelk., 47: 96-102.
9. Huyghebaert, G., F. van Wambeke and G. de Groote, 1984. The effect of pH of diluent, number of spermatozoa and storage method on fertility and hatchability obtained with turkey semen stored for 6 and 24 hours. Arch. Geflugelk., 48: 142-150.
10. Huyghebaert, G., F. van Wambeke and G. de Groote, 1985. The effect of different storage methods of turkey semen on fertility and hatchability. Arch. Geflugelk., 49: 205-211.
11. Lake, P.E., F.L. Cherms and G.J. Wishart, 1984. Effect of aeration on the fertilising ability of turkey semen stored for 48 hours at 5 and 15°C: A study from the 33rd to the 47th week of age. Reprod. Nutr. Develop., 24: 147-153.
12. Pinto, O., D. Amir, H. Schindler and S. Hurwitz, 1985. Fertility of fresh and stored turkey spermatozoa in the presence or absence of glucose or fructose in the suspension medium. Poult. Sci., 64: 1388-1390.
13. Sexton, T.J., 1981. Preservation of turkey semen at 15°C. I. Effect of extender pH and osmotic pressure. Poult. Sci., 60: 1729, abstr.
14. Sexton, T.J., 1981. Preservation of turkey semen at 15°C. II. Effect of sample agitation. Poult. Sci., 60: 1729, abstr.
15. Thurston, R.J., 1985. Semen handling for insemination or storage. Nat'l. Turkey Meeting, Arizona.
16. van Wambeke, F., 1985. New advances in turkey semen preservation. Turkey World., Mar.-Apr.: 32-33.
17. Wishart, G.J., 1981. The effect of continuous aeration on the fertility of fowl and turkey semen stored above 0°C. Brit. Poult. Sci., 22: 445-450.

RESTRICTED FEEDING OF TURKEY BREEDER TOMS

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INTRODUCTION

Previous research (Cecil, 1981, 1984; Krueger et al., 1977; Meyer et al., 1980) has indicated that restricting the body weight gain of breeder toms could result in lower mortality, fewer losses from leg disorders and lower feed costs while maintaining semen production. The use of a low protein feeding regime will restrict body weight gain but toms fed a low protein diet continue to gain weight throughout the breeding season (Waibel and Burke, 1973).

Toms subjected to body weight restriction have been observed to initiate semen production later as compared to full-fed toms (Cecil, 1984; Krueger et al., 1977, 1978; Meyer et al.,1980). Light intensity may influence this sexual development in restricted toms as light intensity of 100 lx improved semen volume and sperm concentration of low weight toms which averaged 12.6 kg at 32 weeks of age (Cecil, 1986).

Three experiments were conducted at the Rosemount Agricultural Experiment Station to determine the effects of restricting body weight of breeder toms (Nicholas male line males) through the feeding of a low protein diet starting at 16 or 20 weeks of age coupled to a feed restriction program to control body weight. In the third study the interaction of light intensity and diet was examined.

METHODS

Experiments 1 and 2. Body weight restriction was begun at 16 weeks of age by feeding a low protein (10%) diet (Table 1). The control toms were full-fed a corn-soy mash diet containing 15.7 and 13.4% protein during 16-20 and 20-52 weeks of age, respectively. When the body weights of the toms fed the low protein diet reached 14.5 kg at 28 weeks of age the intake of diet was limited on a daily basis to maintain body weight in the range of 14.5 to 15.5 kg.

Since the toms were housed in the same building as the hens they were placed on short days (6L:18D) at 20 weeks of age. The toms were light stimulated at 27 weeks of age and the hours of light maintained at 15L:9D for the remainder of the study. Light intensity was 0.5 FC.

Measurements of semen production were made weekly with 5 toms collected from each of the two replicate pens (27 toms/pen) per treatment starting at 32 weeks of age. Volume and sperm cell concentration were measured. To determine fertility, Large white (Nicholas female line) hens of the same age were inseminated weekly with .05 ml pooled semen (extended 1 part semen to 2 parts extender). Fertility and hatchability were determined by incubation of eggs collected during one week of every four week production period from 33 to 53 weeks of age. In Experiment 2 the procedure was as in Experiment 1 except the termination of the study was at 43 weeks of age.

Experiment 3. At 20 weeks of age the toms were randomized and assigned to one of three dietary treatments under two light intensities. The control and low protein diets(Diets 1 and 2, Table 1) were as in Experiments 1 and 2. In addition a low protein diet (8.6%) which was full-fed was included in the test. The two light intensities were low (0.5 FC) and high (3 FC) provided by 11 and 60 watt (dimmed) incandescent bulbs, respectively. Each pen was 8' x 16' with two lamps per pen. The lighting program was 12L:12D from 20-30 weeks of age and 14L:10D from 30 weeks to the end of the study at 60 weeks of age.

On a preliminary testing basis one pen of cull toms at the higher ligh intensity treatment was fed Diet 4 (Table 1) containing 40% peat to gain experience in restricting body weight by feeding a very low energy diet. These toms were left over after the tom pens were set up for the main part of the study and were fed the peat diet starting at 24 weeks of age.

Measurements of body weight, feed intake and semen production (semen samples taken weekly starting at 32 weeks of age from 4 toms per diet-light treatment) were made. Hens were inseminated with semen (.035 ml extended 1:1) and fertility and hatchability were determined on one week's egg collection in each 4 week laying period.

RESULTS

Experiment 1. Performance is summarized in Table 2. Body weight of the restricted toms at 30 and 52 weeks of age averaged 74 and 73% of the control, respectively. Feed intake of the restricted group averaged 77 and 68% of the control during the age periods of 16-30 and 30-52 weeks. Semen production (volume and concentration) was decreased slightly in the restricted toms as compared to the control over the 20 week period of measurement. A statistically significant interaction between age and diet was observed for volume. Restricted toms tended to produce less semen in the latter 10 weeks of the study as compared to the control. Percentage of toms not yielding semen was greater for the restricted toms during the early and later parts of the study. Fertility and hatchability of eggs produced by the test hens were not affected by diet.

Experiment 2. Body weight of restricted toms averaged 65 and 73% of the control full fed toms at 30 and 43 weeks of age (Table 3). Feed intake averaged 73% of the control. Mortality averaged 43.8 and 33.8% for the control and low protein fed toms, respectively.

Statistically significant differences were also observed for volume and fertility. The poor overall values for the restricted group are due primarily to the slow initiation of semen production at time of the first artificial insemination event at 32 weeks of age. Although the toms were on the same lighting schedule as in Experiment 1, feed intake may have been too severely restricted from 27 to 30 weeks as evidenced by the lower body weights at 30 weeks of age as compared to 27 weeks. Also the use of a short day lighting program and low light intensity may have affected initiation of semen production. Sperm number and semen volume in the restricted toms took approximately 4 weeks, to reach the level of the control group.

The slow initiation of semen production in the toms fed Diet 2 was reflected in poor fertility of eggs collected for testing at 36 weeks. Here fertility averaged 94 and 81.5%, respectively, for the control and restricted fed toms. Once full semen production was reached no differences were observed in sperm number, fertility and hatchability. Semen volume tended to remain lower in the restricted toms to the end of the study at 43 weeks.

Experiment 3. The pattern of growth and feed intake for toms fed the Diets 1-3 was similar in the two lighting environments. Feeding the two low protein diets (Diets 2 and 3, Table 1) slowed the growth of the toms (Table 4). Feed restriction of Diet 2 began shortly after 24 weeks of age with feed intake limited in the range of 295-340 g per bird per day. Feed intake of the restricted fed toms (Diet 2) and the low protein fed toms (Diet 3) averaged 74 and 88% of the full fed control group. Mortality for Diets 1, 2, and 3 averaged 25, 6.7 and 15%, respectively.

Semen volume (Table 4) was similar for all 3 diets. Sperm concentration was lowest for the toms fed low protein diets (Diets 2 and 3). No differences in fertility were observed. There was a lower hatch of fertile eggs for the control toms as compared to the lower protein feeding regimes.

For the one pen of toms fed the peat-containing diet, body weight averaged 15.8 kg during 33-60 weeks. Feed intake at 554 g/day was 127% of the control fed toms. Numerically slightly lower sperm numbers were observed. Fertility and hatchability was not measured. Samples taken for semen measurements showed apparent contamination with particles of peat observed microscopically even though care was taken to clean the vent area prior to collection of semen.

The effect of light intensity on reproductive performance is given in Table 5. No differences in fertility or hatchability were observed. Toms housed under low light intensity produced a higher volume and more concentrated semen as compared to those housed under higher light intensity.

SUMMARY

Restriction of tom body weight was accomplished through ad libitum and controlled feeding of low protein diets. Feed intake and mortality was reduced through feeding of low protein diets. Semen volume or sperm concentration were sometimes reduced with low protein diets although sperm number per ejaculate was similar for control and low protein fed toms in Experiments 2 and 3. Restriction of body weight to less than 14.5 kg appeared to affect initiation of semen production as observed in Experiment 2.

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LITERATURE CITED

Cecil, H., 1981. Effects of dietary protein on body weight and reproductive performance of male turkeys. Poultry Sci. 60:1049-1055.

Cecil, H., 1984. Effect of dietary protein and light restriction on body weight and semen production of breeder male turkeys. Poultry Sci. 63:1175-1183.

Cecil, H., 1986. Effect of light intensity during the breeder period on semen production of low weight and normal weight breeder toms. Poultry Sci. 65:1900-1904.

Krueger, K. K., J. A. Owen, C. E. Krueger and T. M. Ferguson, 1977. Effect of feed or light restriction during the growing and breeding cycles on the reproductive performance of Broad Breasted white turkey males. Poultry Sci. 56:1566-1574.

Krueger, K. K., P. S. Kaylor, and D. R. McIntyre, 1978. Compensatory growth in the turkey breeder male and its effect on reproductive performance. Poultry Sci. 57:1149-1150.

Meyer, G. B., C. F. Props, A. T. Leighton, Jr., H. P. Van Krey, and L. M. Potter, 1980. Influence of dietary protein during the pre-breeder period on subsequent reproductive performance of Large White turkeys. 2. Growth, feed consumption, and male sex-limited reproductive traits. Poultry Sci. 59:358-362.

Waibel, P. E. and W. H. Burke, 1973. Nutrition of turkey breeder males. Minn. Turkey Research Report, Univ. of Minnesota Agricultural Experiment Station Misc. Rept. 121, p. 37-42.

Table 1. Composition of diets in Experiments 1,2 and 3.

Ingredient %	DIET TYPE					
	Diet 1		Diet 2		Diet 3	Diet 4
	Control		Low Protein Restricted		Low Protein Ad Lib	Peat
Age Period (weeks):	16-20	20-52	16-20	20-52	20-60	20-60
Corn	76.60	83.78	92.64	93.13	97.14	50.27
Soybean meal, solv.	19.89	13.64	3.69	4.10	-	8.18
Peat	-	-	-	-	-	40.0
Fish solubles product	.40	-	.40	-	-	-
Fermentation residue product	.04	-	.04	-	-	-
Dicalcium phosphate	1.423	1.108	1.521	1.210	1.257	.660
Calcium carbonate	.992	.848	1.054	.91	.946	.510
Salt	.400	.400	.400	.416	.425	.240
Trace mineral mix MNTM	.080	.070	.080	.076	.078	.042
Vitamin mix MTG-74	.175	.150	.175	.155	.157	.090
Nutrient Composition (calculated):						
Protein,%	15.72	13.37	10.00	10.00	8.55	8.02
Metabolizable energy, kcal/kg	3082	3178	3271	3289	3332	1906
Lysine,%	.78	.60	.34	.34	.23	.36
Methionine plus cystine, %	.54	.48	.38	.38	.34	.29

Diets 1 and 2 used in Experiment 1 for specified age period and in Experiment 2 during 16-45 weeks of age.
Diets 1-4 used in Experiment 3 from 20-60 weeks of age.

Table 2. Effect of breeder tom feeding program on body weight, feed intake and reproductive performance, Experiment 1.

	Diet number and description	
	1 Control, full-fed	2 Low protein, restricted
Body weight (kg) [1] at week:		
20	12.3	10.4
27	17.7	13.4
30	19.7	14.6
33	19.8	14.8
43	19.8	14.5
52	20.8	15.3
Feed intake(g/day) [1] for weeks:		
16-30	483	371
30-52	491	334
Semen volume [2] (ml)	.36	.3
Sperm number [2] (billion cells/ml)	10.56	9.81
Fertility (%) [3]	95.6	95.4
Hatch of Fertile (%) [3]	85	83.9

1. Average of two replicate pens (27 toms started per pen).
2. Sample of 5 toms taken per replicate pen.
3. Average of five settings at 4 week intervals during 20 weeks of egg production. Each semen source inseminated 24 hen pens (12/pen).

Table 3. Effect of breeder tom feeding program on body weight, feed intake and reproductive performance, Experiment 2.

	Diet number and description	
	1 Control, full-fed	2 Low protein, restricted
Body weight (kg) [1] at week:		
20	13.9	11.2
27	19.6	14.4
30	21.3	13.8
33	22.6	15.2
43	21.4	15.6
Feed intake(g/day) [1] for weeks:		
16-43	549	399
Semen volume [2] (ml)	.21	.17
Sperm number [2] (billion cells/ml)	7.53	9.22
Fertility (%) [3]	93.8	89.2
Hatch of Fertile (%) [3]	73.4	73.3

1. Average of two replicate pens (40 toms started per pen).
2. Sample of 6 toms taken per replicate pen.
3. Average of three settings at 4 week intervals during 12 weeks of egg production. Each semen source inseminated 24 hen pens (12/pen).

Table 4. Effect of breeder tom feeding program on body weight, feed intake, and reproductive performance averaged over light intensity treatments, Experiment 3.

Diet number and description	Body weight at week: [1] 24	27	30	33	43	60	Feed intake [1] 20-60 wks	Semen Volume [2]	Sperm Number [2]	Fert-ility [3]	Hatch of Fertile [3]
	(kg)						(g/day)	(ml)	(billion/ml)	(%)	(%)
1. Control/ full-fed	16.6	18.4	19.3	20.6	20.7	21	434	.19	11.26	85.9	76.9
2. Low protein, 10%, restricted	14.7	15.2	15.6	16.7	16.7	17.1	323	.21	10.25	87.5	79
3. Low protein, 8.6%, full-fed	14	15.3	15.7	16.2	17.4	19.3	383	.19	10.17	87.5	80.2
4. Peat [4]	--	14.5	14.2	15.7	16.1	15.7	554	.19	10.1	--	--

1. Average of four pens with two replicate pens per each diet-light intensity treatment (15 toms started per pen).
2. Samples taken from 2 toms per pen.
3. Average of seven settings at 4 week intervals during 28 weeks of egg production. Pooled semen from each diet-light intensity was used to inseminate 8 pens of hens (12/pen).
4. Data from 1 pen at high light intensity from 24-60 weeks of age.

Table 5. Effect of light intensity on reproductive performance of breeder toms, averaged over diet, Experiment 3.

Light intensity (FC)	Semen Volume[1]	Sperm Number[1]	Fertility[2]	Hatch of Fertile[2]
	(ml)	(billion per ml)	(%)	(%)
Low, .5	.205	11.34	87.3	78.7
High, 3	.182	9.75	86.6	78.8

1. Average of six pens with samples taken from 2 toms per pen.
2. See footnote 2, Table 4.

CIRCULATING PROLACTIN: AN INDEX OF BROODY MANAGEMENT PROGRAM EFFECTIVENESS

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The loss of potential egg production and the added labor to conduct a successful management program to discourage hens from becoming broody are costly to producers of hatching eggs. If broody hens are not treated in a manner to interrupt broodiness, many hens will continue to be broody for a long time. The longer broodiness is allowed to persist, the more complete will be the associatd ovarian regression. For this reason, early detection and interruption of broodiness is commercially critical in that if broodiness is terminated expediently, substantial ovarian regression will not have occurred and the hens will usually continue egg production. Alternatively, if broodiness is allowed to persist for several days prior to interruption attempts, broodiness associated ovarian regression will prevent economically important return to egg production.

A variety of techniques have been investigated and few have been used commercially. The technique most used commercially involves moving the potential broody hens from their familiar places to a strange and less comfortable place without nests such as broody coop, wire floored pen or cages. Other less known approaches have involved the application of strong sensory stimuli such as high intensity sound and electric shock. However, high light intensity and/or continuous light are used commercially in some instances. A third approach involves the administration of ovarian steroids, i.e., progesterone and diethylstilbestrol.

These techniques require expertise in order to diagnose the onset of broodiness before ovarian regression begins. Thus, early detection and treatment of broody turkeys is necessary to maintain efficient egg production.

Identification of broody hens is dependent on detection of the increased nesting activity. The return of a hen to the trap-nest 3-4 times per day on 2 consecutive days when forced from the trap-nest 6-7 times per day is considered an early indication of broodiness. When open nest or semi-trap nest are used, hens found on the nest at the end of the photophase on 2 consequitive days are considered broody. Due to the expertise needed to identify and the labor required to remove broody hens, the whole flock may be moved to a nonfamiliar place at different time intervals, about once every 10 days. This can be accomplished by rotating the flocks between different buildings or between pens within the same building. Another management precedure used by breeder men and women to discourage the initiation of broodiness is the closing of nests at the end of the photophase and reopening them the next morning.

It is now clear that the hormone prolactin is capable of inducing both broodiness and ovarian regression and termination of egg production. It is also clear that management programs known to disrupt broodiness also lower circulating prolactin levels.

The objective of this study was to evaluate the management programs used for broody treatments in the field. This is accomplished by measuring circulating

prolactin levels before and after the application of broody treatments.

The broody programs for 13 breeder flocks were evaluated. The programs used included: (1) identification of broody hens and moving them to broody pens (8 flocks); (2) as (1) in addition to 24 hours of light (2 flocks); (3) whole flock rotation between 2 building (1 flock); and (4) whole flock rotation between pens in the same building (3 flocks).

Each farm was provided with a kit equipped with instruction and materials needed for blood collection. Blood samples were collected from hens between 6-8 weeks of egg production.

In broody programs that utilized the identification and removal of broody hens from the flock and their return at the end of treatment, blood samples were obtained immediately before the initiation of broody treatment and at the end of treatment. In programs designed to discourage broodiness by rotating the whole flock with no special attention to individual hen, blood samples were collected from hens found on the nest immediately before rotating and 5 days after rotation. In all the programs, sister laying hens were used as controls.

The results of the main effects are presented in Table 1. Serum prolactin levels of hens identified as broodies before removal for treatment were 234.9±21.5 ng/ml. Following treatment, the levels were reduced to 99.8±10.5 ng/ml which is essentially similar to those of their corresponding laying hens

Table 1. Effect of broody treatment on serum prolactin levels (ng/ml).

Treatment Type	Broodies or birds found on the nest		Layers	
	Before Treatment	After Treatment	Before Treatment	After Treatment
Identification and removal to broody pens	234.9±21.5 *F = 8 *N = 76	99.8±10.5 F = 8 N = 81	119.6± 9.1 F = 8 N = 79	104.8± 9.1 F = 8 N = 77
Identification and removal to broody pens plus 24 hr light	119.5±17.1 F = 2 N = 20	98.8±16.4 F = 2 N = 20	115.0±11.9 F = 2 N = 20	144.6±28.8 F = 2 N = 20
Rotation within building	186.5±28.9 F = 3 N = 30	135.1±15.2 F = 3 N = 31	97.4±12.9 F = 3 N = 30	82.1±12.2 F = 3 N = 29
Rotation between buildings	54.2± 6.9 F = 1 N =10	80.2±21.4 F = 1 N = 10	81.2±19.5 F = 1 N = 10	47.4±11.3 F = 1 N = 9

*F = number of flocks
N = number of birds

(119.6±9.1 ng/ml and 104.8±9.1 ng/ml). Treatment of broody hens with an additional 24 hours of light does not appear to have further effect on prolactin level (98.8±16.4 ng/ml vs. 99.8±10.5 ng/ml).

The overall mean of circulating prolactin of laying hens and those treated for broodiness was 100.5±7.6 ng/ml. This value was used to determine the effectiveness of broody treatments on each farm. The percent of hens identified as broodies ranged from 30% to 100% with an average of 74.1±8.7%. That is about 26% of hens thought to be broodies were layers.

There were total of 4 flocks in which the rotation method was used. In three of the flocks, rotation was performed between pens in the same building. One of these three flocks had its pretreatment circulating prolactin low (79.9 ± 6.0 ng/ml) compared to the average pretreatment of the other two flocks (239.9 ± 39.0 ng/ml). This may be explained by the frequency of rotation. The greater the frequency the lower is the PRL level. Even though a limited number of rotation flocks were available for the comparison, it appears that rotation between buildings was more effective than rotation within the building. Rotation between buildings appears to lower circulating prolactin levels in all birds including the layers.

5-AZACYTIDINE INDUCED INCREASE IN PLASMA PROLACTIN IN IMMATURE TURKEYS

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INTRODUCTION

Turkey reproductive efficiency is low in comparison to that of chickens. The relatively limited egg production of turkeys is a major component of this low efficiency. In turn, the low egg production is caused by the propensity of turkeys toward early cessation of egg laying and subsequent onset of maternal behavior (broodiness). Broodiness in turkeys and chickens has long been correlated with increased prolactin (Prl) secretion. The broody condition which follows a period of egg laying, is characterized by dramatically increased serum Prl levels and decreased levels of the circulating reproductive hormones; luteinizing hormone (LH), estradiol and progesterone (Cogger et al., 1979; Burke and Dennison, 1980; El Halawani et al., 1980; Goldsmith and Williams, 1980; Bedrak et al., 1981; Proudman and Opel, 1981). Broodiness is also associated with specific behavioral and physiological events--aggressive nest protection, cessation of ovulation and ovarian regression (Collias, 1950; El Halawani et al., 1980; El Halawani et al., 1983).

Recent work has produced evidence implicating increased Prl secretion as a cause for the ovarian regression associated with the shift from egg laying to broodiness (El Halawani et al., 1983; El Halawani et al., 1984). Opel and Proudman (1980) were able to induce ovarian regression in both turkeys and chickens with exogenous ovine Prl. Radioimmunoassay for avian Prl has allowed demonstration of a marked increase in circulating Prl during broodiness and its associated ovarian regression.

Increased synthesis of a protein such as Prl frequently requires adjustments in regulation at both the level of translation and transcription. There has been extensive work correlating the regulation of specific gene expression and the state of methylation of cytosine residues in the DNA encoding that gene. The pattern that has emerged correlates hypomethylation with increased gene activity and hypermethylation with suppressed activity (reviews Riggs, 1975; Razin and Riggs, 1980).

While methylation-demethylation is not the only mode of transcriptional control of gene expression, demethylation often appears to be an initial event in the induction of increased gene activity. Other mechanisms, as yet undefined, then apparently assume primary control of the induced synthesis. The state of methylation of cytosines in cytosine-guanine pairs in DNA is maintained through the action of the enzyme hemimethylase, which recognize partially methylated sites created due to semi-conservative DNA replication or DNA repair. In order to duplicate the pre-existing methylation pattern in the daughter cells or newly repaired DNA, the hemimethylase recognizes partially methylated sites and catalyses the addition of a methyl group to the newly added cytosine. 5-azacytidine is a cytidine analog that has been shown to have the ability to inhibit the enzymes that methylate cytosine residues in eukaryotic DNA. Although the drug acts as a general inhibitor of hemimethylase, it has been shown to activate only selective genes in a system rather than the expected universal increase in gene expression (review Jones, 1985) indicating, again other levels of expression

control beyond methylation.

The work presented here utilizes this hemimethylase inhibiting activity of 5-azacytidine to investigate the role of methylation in the control of Prl gene expression in the domestic turkey.

MATERIALS AND METHODS

Sexually immature (7-weeks-old) female domestic turkeys of the Nicholas strain were used in these experiments. The birds were housed in floor pens and maintained under a 15 hr light:9 hr dark photoregimen, with lights on at 0600 hr. Feed and water were constantly available. The drugs, dissolved in 0.9% NaCl (saline), were administered by 0.5 ml intravenous injection, with saline injected as a control. The drug dosage, in milligrams per kilogram total body weight, was based on the average weight of the birds within the particular treatment groups at the initiation of the experiment. Drug solutions were prepared immediately prior to the beginning of each treatment. Blood samples (2 ml) were collected by venipuncture of the brachial vein. The Day 1 bleedings were done immediately prior to drug injections. The plasma was separated by centrifugation, harvested, and stored at -20°C until assayed. The plasma samples were assayed for Prl content using the homologous radioimmunoassay described by Proudman and Opel (1981).

In experiment 1, 5-azacytidine (5 mg/kg; Sigma Chemical Co.) treatment occurred on days 1-6, 15-18, and 28-31. Blood samples were collected immediately prior to drug injection on days 1, 6, 10 and 31. In experiment 2, birds were treated with 5-azacytidine, cytidine, 6-azathymine (all at 5.0 mg/kg; Sigma), or saline on days 1-6. Blood samples were collected immediately prior to drug injection on days 1, 3, 5, 7, and 10.

Data were analyzed by analysis of variance with a split-plot design using the ANOVA procedure of the Statistical Analysis System (Helwig and Council, 1979). Duncan's multiple range test was used to compare means.

RESULTS

In experiment 1, 5-azacytidine treatment induced a significant elevation in Prl levels ($P<0.006$), compared to those of the saline treated group (see Fig. 1). Prior to treatment, Prl levels in both treatment groups were similar ($P>0.75$). Within the 5-azacytidine treatment group Prl levels were significantly greater ($P<0.05$) on day 6 than on any of the other sampling days.

In experiment 2, 6-azathymine or cytidine treatment did not alter Prl levels from that observed in the saline treated group ($P>0.44$; Fig 2A). 5-azacytidine treatment elevated circulating Prl levels ($P<0.009$) over that observed in the saline treated groups (Fig. 2B). Within the 5--azacytidine treatment group Prl levels were significantly greater ($P<0.05$) on days 5 and 7 than on any of the other sampling days.

DISCUSSION

5-azacytidine treatment of immature turkey hens induced a significant increase in plasma Prl levels. This response was not seen in the saline injected controls, nor in those birds treated with cytidine or 6-azathymine. The non-response to the nucleoside cytidine and the aza analog of thymine reasonably eliminates possible non-specific drug stress as the cause of the Prl increase--a necessary consideration given the noted increases in plasma Prl levels observed with certain forms of stress (El Halawani et al., 1985).

The pattern of response seen--a rapid increase in Prl plasma levels and then a decline back to near basal levels--is consistant with responses seen in other systems. Most notably, it parallels the elicitation of transient expression of fetal hemoglobin upon 5-azacytidine treatment of human patients with sickle cell anemia and thalassemia (Lay et al., 1982; Charache et al., 1983). This rise and fall of plasma Prl levels seems to indicate that while demethylation may switch on the expression of Prl, other mechanisms must maintain that expression. Since the turkeys used in our work were immature birds, those necessary maintenance mechanisms may not be developed. This may also explain the relatively low level of enhanced Prl release observed here; which, while still significant, is an order of magnitude less than that observed in a broody bird. The interaction of multiple control systems of gene expression upon 5-azacytidine treatment has been noted previously in chickens by Ginder et al. (1984), where butyrate was needed in conjunction with 5-azacytidine to elicit the transient expression of embryonic globin. The necessity for a secondary mechanism for Prl expression in turkeys is currently being investigated by our group through 5-azacytidine treatment of sexually mature hens.

Thus we have shown a marked increase in plasma Prl levels in turkeys treated with the known demethylating agent, 5-azacytidine. This supports the concept that Prl may be under methylation control, as was indicated in the study of Durrin et al. (1984) where the Prl gene in Prl-expressing pituitary tumor cells of Fischer 344 rats was found to be hypomethylated when compared to the Prl gene of the non-expressing rat liver. Based on our results further examination of the role of methylation in Prl expression in turkeys is warranted.

ACKNOWLEDGEMENTS

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REFERENCES

Bedrak, E., S. Harvey and A. Chadwick, 1981. Concentrations of pituitary, gonadal and adrenal hormones in serum of laying and broody white rock hens (Gallus domesticus). J. Endocrinol., 89: 197-204.

Burke, W.H. and P.T. Dennison, 1980. Prolactin and luteinizing hormone levels in female turkeys (Meleagris gallopavo) during the photo-induced reproductive cycle and broodiness. Gen. Comp. Endocrinol., 41: 92-100.

Charache, S., G. Dover, K. Smith, C.C. Talbot, Jr., M. Moyer and S. Boyer, 1983. Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the δ-ß-globin gene complex. Proc. Natl. Acad. Sci., U.S.A., 80: 4842-4846.

Collias, N.E., 1950. Hormones and behaviour with special reference to birds and the mechanism of hormone action. In: A symposium on steroid hormones (E.S. Gordon, ed.). University of Wisconsin Press, Madison, WI, pp. 277-329.

Cogger, E.A., W.H. Burke and L.A. Ogren, 1979. Serum luteinizing hormone, progesterone and estradiol levels in relation to broodiness in the turkey (Meleagris gallopavo). Poultry Sci., 58: 1355-1360.

Durrin, L.K., J.L. Weber and J. Gorski, 1984. Chromatin structure, transcription, and methylation of the prolactin gene domain in pituitary tumors of Fischer 344 rats. J. Biol. Chem., 259: 7086-7093.

El Halawani, M.E., W.H. Burke and P.T. Dennison, 1980. Effect of nest-deprivation on serum prolactin levels in nesting female turkeys. Biol. Reprod., 23: 118-123.

El Halawani, M.E., J.L. Silsby, E.J. Behnke, and S.C. Fehrer, 1984. Effect of ambient temperature on serum prolactin and luteinizing hormone levels during the reproductive life cycle of the female turkey (Meleagris gallopavo). Biol. Reprod., 30: 809-815.

El Halawani, M.E., J.L. Silsby, S.C. Fehrer and E.J. Behnke, 1983. Reinitiation of ovulatory cycles in incubating female turkeys by an inhibitor of serotonin synthesis, p-chlorophenylalanine. Biol. Reprod., 28: 221-228.

El Halawani, M.E., J.L. Silsby, S.C. Fehrer and E.J. Behnke, 1985. The influence of acute or repeated immobilization on plasma prolactin levels in the turkey (Meleagris gallopavo). Gen. Comp. Endocrinol., 59: 410-415.

Ginder, G.D., M.J. Whitters and J.K. Pohlman, 1984. Activation of a chicken embryonic globin gene in adult erythroid cells by 5-azacytidine and sodium butyrate. Proc. Natl. Acad. Sci. U.S.A., 81: 3954-3958.

Goldsmith, A.R. and D.M. Williams, 1980. Incubation in mallards (Anas platyrhynchos): Changes in plasma levels of prolactin and luteinizing hormone. J. Endocrinol, 86: 371-379.

Helwig, J.T. and K.A. Council (editors), 1979. Statistical Analysis System User's Guide. University of North Carolina, Raleigh, NC:SAS Institute.

Jones, P.A., 1985. Altering gene expression with 5-azacytidine. Cell, 40: 485-486.

Ley, T.J., J. Simone, N.P. Anagnou, G.H. Keller, R.K. Humphries, P.H. Turner, N.S. Young, P. Heller and A.W. Nienhuis, 1982. 5-azacytidine selectively increases γ-globin synthesis in a patient with ß+ thalassemia. N. Eng. J. Med., 307: 1469-1475.

Proudman, J.A. and H. Opel, 1981. Turkey prolactin: Validation of a radioimmunoassay and measurement of changes associated with broodiness. Biol. Reprod., 25: 573-580.

Razin, A. and A.D. Riggs, 1980. DNA methylation and DNA function. Science, 210: 604-610.

Riggs, A.D., 1975. X inactivation, differentiation and DNA methylation. cytogenet. Cell. Genet., 14: 9-25.

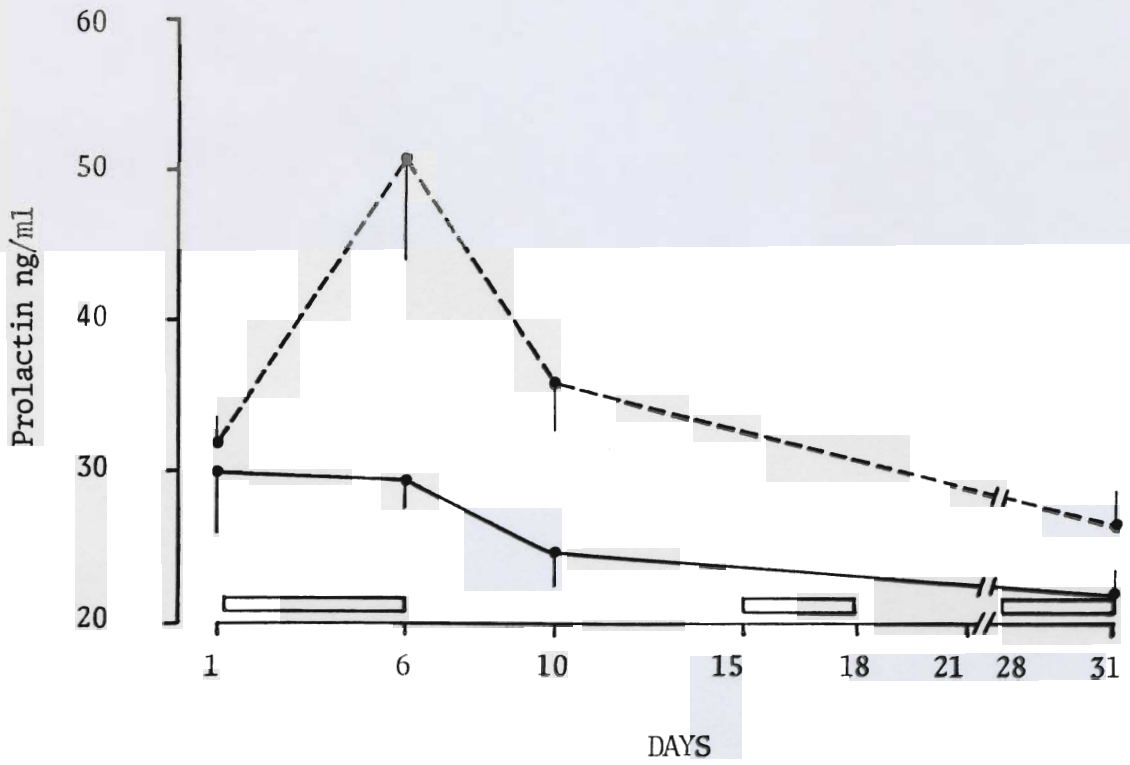
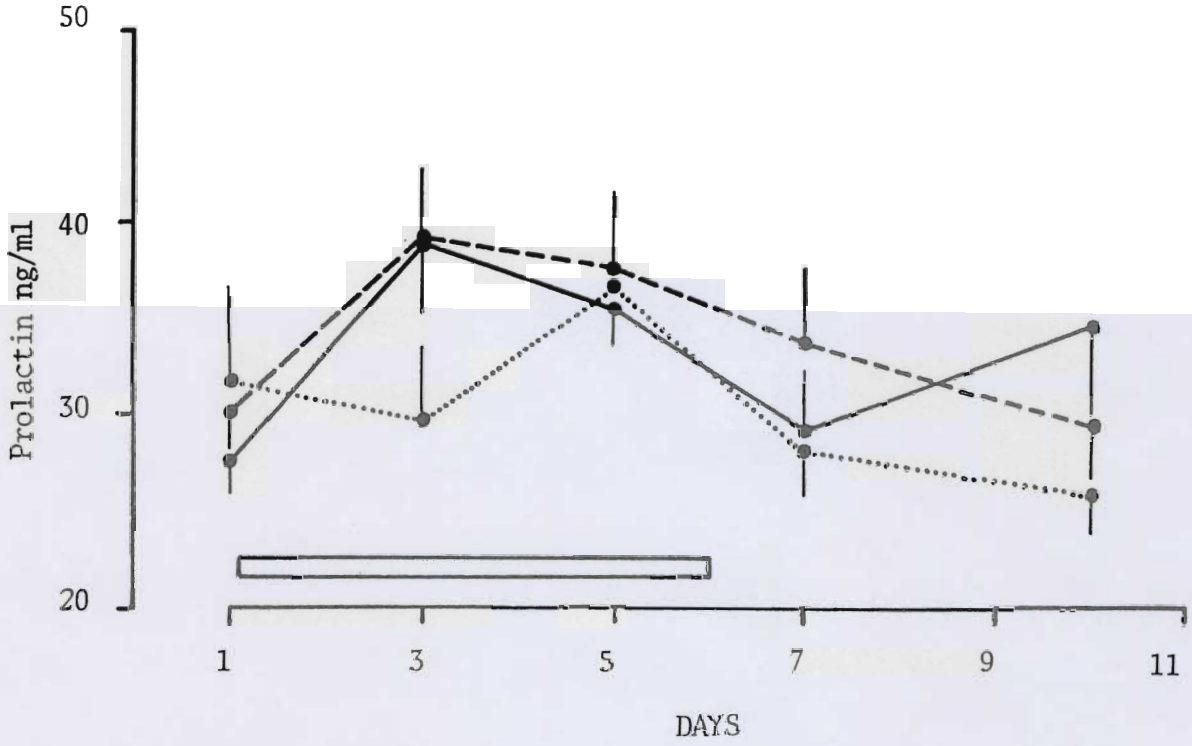


Fig. 1. Effect of periodic intravenous 5-azacytidine administration on the prolactin level of immature turkeys. Each point represents the mean and the vertical line represents the standard error of the mean.



Saline Treated

6-azathymine Treated

Cytidine Treated

Treatment Period

Fig. 2A. Effect of daily intravenous administration of cytidine or 6-azathymine on the prolactin level of immature turkeys.

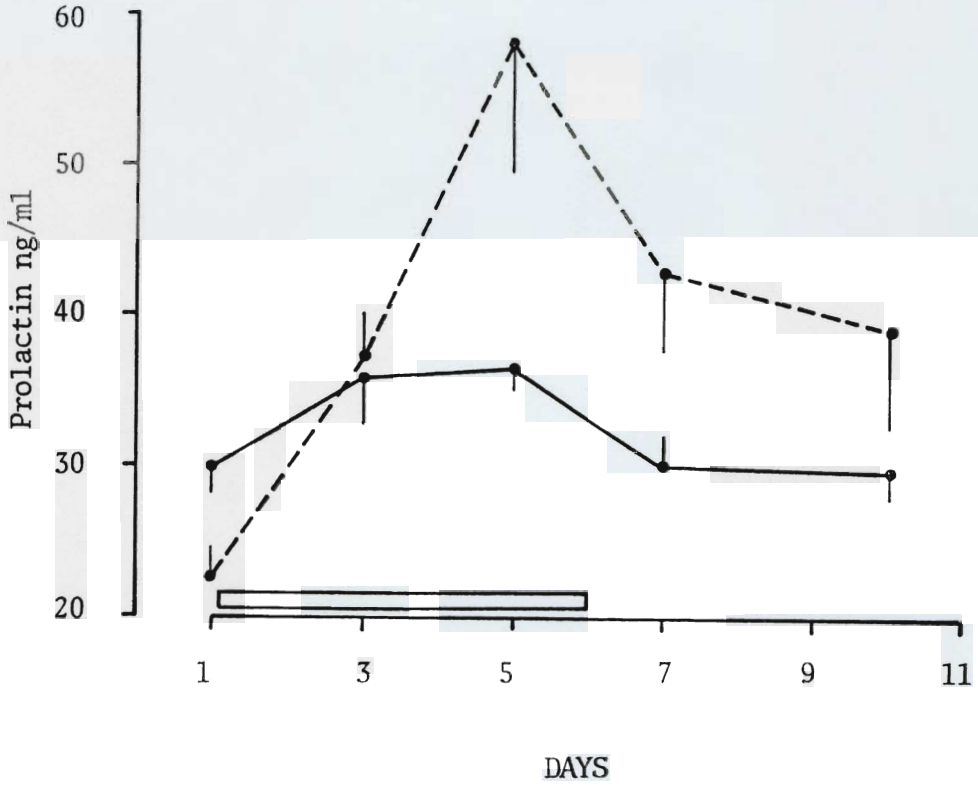


Fig. 2B. Effect of daily intravenous administration of 5-azacytidine on the prolactin level of immature turkeys.

UTILIZATION OF FEATHER MEAL, BLOOD MEAL, AND MEAT MEAL IN MARKET TURKEY DIETS

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Feather meal, blood meal, and meat and bone meal have long been recognized as principal animal byproducts for use in rations of poultry. As might be expected, where processing involving heat is so important to the production of a product, quality of these products has been variable. Nutritionists have not always been willing to commit valued feeding programs to open use of these products.

The turkey nutrition program at this University recently completed two market studies utilizing products believed to be of high quality. The feather meal (hydrolyzed ring dried) and meat and bone meal (Gro-mor Gold) were provided by Central Bi-Products, Redwood Falls, MN. The blood meal (ring dried) was provided by Van Hoven Co., South St. Paul, MN.

There have been many questions about the detailed results and feed formulations used. The purpose of this paper is to make this information available.

The nutrient content of the ingredients used in diet formulation is presented in Table 1. All diets contained 2% supplemental fat and were fed in the meal form. Nutrient levels were taken or adapted from National Research Council (1984) requirement levels, and were utilized on a per therm of metabolizable energy basis. Methionine, methionine + cystine, and lysine levels were the same as NRC and calcium and phosphorus were 1.10 times NRC.

The meat level was held at 7% throughout both experiments. Feather and blood meal levels were at 1.0, 0.9, 0.8, 0.7, and 0.6 times the starting (0-4 wk) level in successive feeding periods.

Both studies were conducted in 50 pens (each 6' x 8 ') of the Nutrition Building of the Rosemount Agricultural Experimentation Station. There were 10 treatments, with 5 replicate pens per treatment. Turkeys were of the Nicholas strain.

STUDY 1. TOMS (EXPERIMENT TG-862)

The study was begun January 13, 1986 and terminated June 3, 1986, when the turkeys were 20 weeks of age. Average building temperatures in successive 4 week periods were 70, 64, 64, 67, and 71 degrees F.

Detailed diet composition is presented in Table 2. Supplemental lysine was not allowed to enter the diets; so, in order to meet the lysine requirement with feather meal it was necessary to concomitantly increase dietary protein.

Brief descriptions of the treatments accompany the growth data in Table 3. Treatments 1-7 all are of "normal" protein content. Treatments 8-10 were formulated to contain 90% of the NRC lysine , methionine, and methionine + cystine levels. Feed efficiency data are provided in Table 4.

Where products were added directly to the corn-soybean meal diet of NRC protein level (trt 1), feather meal at 2% (trt 2) or 4% (trt 3) resulted in 1 or 2% growth reductions with no change in feed efficiency (differences not significant). Meat and bone meal at 7% of the diet (trt 4) resulted in similar body weight gains; here the improvement in feed efficiency was nearly significant.

The next comparison set includes treatments 4-7, all containing 8% meat and bone meal. At 4 weeks of age, the presence of 4% feather meal resulted in reduced gains, either with or without blood meal. The only other significant growth difference was a depression with blood meal (alone) at 16 weeks of age. There were no significant differences in growth or efficiency of feed utilizaton at 20 weeks of age.

The final comparison set is with the lower protein level. Growth was significantly depressed with the low protein corn-soy diet (trt 8) only at 12 and 20 weeks of age. Body weights with feather meal (trt 9) were generally lower than control (trt 8); however, the difference was significant only at 4 weeks of age. Another treatment (trt 10) utilized the same feather meal but its amino acid composition was corrected by available amino acid data of Liu (1986). There was no statistical difference between how the feather meals (trts 9 and 10) related to control (trt 8) or how feather meals of differing amino acid value related to each other (trts 9 and 10); however, body weights were greater (numerically) when feather meal's amino acid composition was adjusted downward.

In general, the byproducts performed very well in this study. General suggestions would be to utilize blood meal at a lower level (perhaps starting at 2% of the diet) and to use feather meal composition based upon available amino acids.

STUDY 2. HENS (EXPERIMENT TG-865)

The study was begun October 2, 1986 and terminated January 28, 1987, when the turkeys were 17 weeks of age. Average building temperatures during 0-4, 4-8, 8-11, 11-14 and 14-17 weeks of age were 74, 66, 62, 61, and 59 degrees F.

Detailed diet composition is presented in Table 5. One major difference in formulation was to allow use of supplemental lysine to accompany feather meal so as to maintain comparable protein levels.

Brief descriptions of the treatments accompany the growth data in Table 6. Treatments 1-8 all are of "normal" protein content. Treatments 9-10 were formulated to contain 85% of the NRC lysine and methionine + cystine levels. Feed efficiency data are provided in Table 7.

The first comparison set (trts 1-4) is of corn-soy diets of NRC protein level and direct substitutions therein. In trts 2 or 3, feather meal starting at 4 weeks of age or at day of age resulted in virtually identical performance, very similar to trt 1 control. In trt 4, meat and bone meal resulted in almost identical body weight gain performance as control; however, efficiency of feed utilization was much improved.

The next comparison set (trts 4-7) includes all diets containing 7% meat and bone meal. In this study blood meal was used at a lower level. Performance was virtually identical for the 4 treatments containing neither, either, or both feather meal and blood meal.

The last comparison is with treatments 9 and 10, containing 85% of the methionine + cystine and lysine levels of NRC (1984). In comparing trts 1 and 9 (both corn-soy), body weight was depressed significantly with low protein through 14 weeks of age. Between 14 and 17 weeks of age the absolute body weight difference closed from 0.26 kg to 0.14 kg (latter difference not significant). The results with treatment 10 were not good. With this lower protein regime, the presence of feather meal depressed body weight gains significantly.

In general, the byproducts again performed very well. Blood meal at 62.5% of the levels in the earlier experiment seemed to perform optimally. The depressing effect of feather meal at the low protein level suggests that a third amino acid may have been limiting.

LITERATURE CITED

Liu, J., 1986. Nutritional evaluation of blood meal and feather meal. M.S. thesis, University of Minnesota.

TABLE 1. Nutrient composition of ingredients. Note description in footnote.

	Corn, ground yellow	Soybean meal, dehulled	Fish solubles product	Fermentation res. product	Feather meal Regular	Feather meal Avail.	Blood meal	Meat and bone meal
Crude protein, %	**8.8**	**47.0**	**53.5**	**23.6**	**81.9**	**81.9**	**90.3**	**53.3**
Metab. energy (kcal/kg)	**3430**	**2440**	**2575**	**2493**	**2360**	**2360**	**3420**	**2549**
Calcium, %	.02	.27	.45	.37	.33	.33	.06	8.05
Phosphorus, available, %	.08	.19	.86	.28	.55	.55	.09	4.35
Crude fat, %	3.8	1.0	8.2	8.0	5.0	5.0	.8	10.7
Crude fiber, %	2.2	3.9	3.9	7.8	.75	.75	.45	2.10
Arginine, %	.50	3.57	3.25	1.09	5.71	5.33	4.22	3.67
Glycine, %	.37	2.22	4.56	1.19	6.47	5.43	4.07	6.60
Serine, %	.40	2.80	2.06	1.37	8.78	8.18	4.93	2.15
Glycine + serine, %	.77	5.02	6.62	2.56	15.25	13.61	9.00	8.75
Histidine, %	.20	1.28	2.14	.64	1.11	.83	5.83	1.11
Isoleucine, %	.37	2.49	2.25	1.00	3.86	3.54	1.31	1.56
Leucine, %	1.10	3.70	3.12	2.90	6.92	6.07	11.70	3.24
Lysine, %	.24	3.08	3.20	.88	2.19	1.60	8.52	2.85
Methionine, %	.20	.70	.83	.69	.58	.50	1.19	.73
Cystine, %	.15	.71	.65	.35	4.39	3.12	1.28	.66
Methionine + cystine, %	.35	1.41	1.47	1.04	4.97	3.62	2.47	1.39
Phenyalanine, %	.47	2.05	2.07	1.19	4.43	4.04	6.53	1.90
Tyrosine, %	.45	1.95	1.04	.85	2.79	2.35	3.14	1.35
Phenylal. + tyrosine, %	.92	3.99	3.11	2.04	7.22	6.39	9.67	3.25
Threonine, %	.39	1.85	1.77	1.29	3.72	3.04	4.34	1.73
Tryptophan, %	.09	.65	.62	---	.50	.42	1.34	.28
Valine, %	.52	2.64	2.33	1.56	6.16	5.28	7.78	2.26

Composition of corn, soybean meal, and fish solubles product was adapted from NRC(1984) "Nutrient Requirements of Poultry". Ferm. residue product values were from company literature. Fish solubles product was Dynasol-100S (Pacific Molasses, St. Paul, MN). Fermentation residue product was Fermacto-500 (Borden, Pet-Ag Div., Hampshire, IL). Feather meal (hydrolyzed ring dried) and meat and bone meal (Gro-mor Gold) were provided by Central Bi-Products, Redwood Falls, MN. Blood meal (ring dried) was provided by Van Hoven Co., So. St. Paul. Protein, fat, fiber, and amino acids not including tryptophan were analyzed values for feather meal, blood meal, and meat and bone meal.

TABLE 2. Diet composition in Experiment TG-862 (Males)

Ingredients No.	1	2	3	4	5	6	7	8	9	10
0-4 weeks:										
Corn, gr. yellow	43.1	42.7	42.2	43.6	42.7	49.2	48.3	47.9	47.0	46.3
Soybean meal, deh.	46.8	45.3	43.9	41.4	38.5	31.4	28.6	41.8	38.9	39.7
Fish sols. product	2.56	2.56	2.56	2.62	2.62	2.70	2.70	2.60	2.60	2.59
Ferm. res. product	.256	.256	.256	.262	.262	.270	.270	.260	.260	.259
Feather meal, hyd.	---	2	4	---	4	---	4	---	4	4
Meat + bone meal	---	---	---	7	7	7	7	---	---	---
Blood meal, ring	---	---	---	---	---	4	4	---	---	---
Dicalcium phosphate	2.84	2.80	2.75	1.33	1.24	1.49	1.40	2.93	2.83	2.82
Calcium carbonate	1.60	1.59	1.61	1.05	1.08	1.22	1.16	1.62	1.65	1.64
DL-methionine (99%)	.224	.144	.102	.224	.099	.277	.126	.185	.080	.078
Salt	.31	.28	.24	.19	.12	.18	.11	.32	.25	.24
Trace min. mix MNTM	.124	.123	.122	.120	.119	.112	.110	.129	.127	.127
Vitamin mix. MTS-74	.256	.256	.256	.262	.262	.270	.270	.260	.260	.259
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	27.4	28.3	29.1	28.7	30.4	28.2	29.9	25.5	27.2	27.5
Met. en., kcal/kg	2851	2849	2846	2916	2911	3004	2999	2896	2890	2884
4-8 weeks:										
Corn, gr. yellow	48.3	47.9	47.5	48.9	48.0	54.2	53.4	52.9	52.0	51.4
Soybean meal, deh.	43.4	42.0	40.7	37.9	35.3	28.6	26.0	38.7	36.0	36.7
Fish sols. product	1.85	1.84	1.84	1.89	1.88	1.94	1.94	1.87	1.87	1.86
Ferm. res. product	.185	.184	.184	.189	.188	.194	.194	.187	.187	.186
Feather meal, hyd.	---	1.9	3.7	---	3.7	---	3.7	---	3.7	3.7
Meat + bone meal	---	---	---	7	7	7	7	---	---	---
Blood meal, ring	---	---	---	---	---	3.7	3.7	---	---	---
Dicalcium phosphate	2.26	2.22	2.18	.75	.65	.87	.78	2.33	2.25	2.23
Calcium carbonate	1.31	1.33	1.34	.76	.81	.84	.87	1.35	1.38	1.37
DL-methionine (99%)	.101	.037	.036	.098	.032	.144	.055	.071	.021	.020
Salt	.31	.28	.25	.20	.13	.18	.11	.32	.25	.25
Trace min. mix MNTM	.113	.113	.112	.110	.108	.102	.100	.118	.117	.116
Vitamin mix MTS-74	.205	.205	.205	.210	.209	.216	.215	.208	.208	.207
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	25.8	26.6	27.4	27.0	28.6	26.5	28.1	24.0	25.6	25.9
Met. en., kcal/kg	2927	2924	2920	2993	2987	3077	3071	2970	2963	2957
8-12 weeks:										
Corn, gr. yellow	56.2	55.9	55.5	57.0	56.3	61.7	60.9	60.2	59.4	58.9
Soybean meal, deh.	36.8	35.7	34.6	31.2	29.0	23.3	21.1	32.8	30.6	31.1
Fish sols. product	1.13	1.13	1.13	1.15	1.15	1.18	1.18	1.14	1.14	1.13
Ferm. res. product	.113	.113	.113	.115	.115	.118	.118	.114	.114	.113
Feather meal, hyd.	---	1.6	3.1	---	3.1	---	3.1	---	3.1	3.1
Meat + bone meal	---	---	---	7	7	7	7	---	---	---
Blood meal, ring	---	---	---	---	---	3.1	3.1	---	---	---
Dicalcium phosphate	1.86	1.83	1.79	.33	.26	.43	.36	1.92	1.84	1.84
Calcium carbonate	1.14	1.15	1.17	.60	.63	.65	.68	1.17	1.19	1.19
DL-methionine (99%)	.023	.003	.002	.018	---	.055	.015	---	---	---
Salt	.31	.28	.25	.19	.14	.18	.13	.32	.26	.26
Trace min. mix MNTM	.103	.102	.102	.099	.098	.092	.091	.107	.106	.105
Vitamin mix MTG-74	.256	.256	.256	.262	.262	.268	.268	.260	.259	.258
Animal fat	2	2	2	2	2	2	2	2	2	2

Table 2. Diet composition in Experiment TG-862 (Males) -- Continued

	1	2	3	4	5	6	7	8	9	10
Crude protein, %	22.9	23.6	24.3	24.1	25.5	23.9	25.3	21.6	23.1	23.0
Met. en., kcal/kg	3019	3016	3013	3088	3081	3133	3132	3037	3029	3044
12-16 weeks:										
Corn, gr. yellow	67.0	66.7	66.4	68.0	67.4	72.3	71.6	70.2	69.5	69.0
Soybean meal, deh.	26.8	25.9	24.9	21.0	19.1	13.9	11.9	23.6	21.7	22.2
Fish sols. product	.77	.77	.77	.79	.79	.80	.80	.78	.78	.77
Ferm. res. product	.077	.077	.077	.079	.079	.080	.080	.078	.078	.077
Feather meal, hyd.	---	1.3	2.7	---	2.7	---	2.7	---	2.7	2.7
Meat + bone meal	---	---	---	7	7	7	7	---	---	---
Blood meal, ring	---	---	---	---	---	2.7	2.7	---	---	---
Dicalcium phosphate	1.64	1.62	1.58	.103	.041	.188	.127	1.68	1.62	1.61
Calcium carbonate	1.05	1.06	1.07	.51	.53	.55	.57	1.07	1.09	1.09
DL-methionine (99%)	.035	.004	.003	.031	---	.063	.014	.009	---	---
Salt	.31	.29	.26	.20	.15	.18	.13	.32	.27	.27
Trace min. mix MNTM	.093	.092	.092	.089	.088	.082	.081	.096	.095	.094
Vitamin mix MTG-74	.205	.205	.205	.210	.209	.214	.214	.207	.207	.206
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	19.0	19.5	20.2	20.0	21.3	19.6	20.8	17.7	18.9	19.1
Met. en., kcal/kg	3134	3132	3129	3206	3200	3271	3266	3165	3158	3154
16-20 weeks:										
Corn, gr. yellow	74.8	74.5	74.2	75.9	75.3	79.9	79.3	77.4	76.8	76.3
Soybean meal, deh.	19.9	19.0	18.2	13.9	12.2	7.4	5.7	17.2	15.5	16.0
Fish sols. product	.41	.41	.41	.42	.42	.43	.43	.41	.41	.41
Ferm. res. product	.041	.041	.041	.042	.042	.043	.043	.041	.041	.041
Feather meal, hyd.	---	1.2	2.4	---	2.4	---	2.4	---	2.4	2.4
Meat + bone meal	---	---	---	7	7	7	7	---	---	---
Blood meal, ring	---	---	---	---	---	2.4	2.4	---	---	---
Dicalcium phosphate	1.39	1.36	1.33	---	---	---	---	1.42	1.36	1.36
Calcium carbonate	.94	.95	.96	.31	.30	.38	.37	.96	.98	.97
DL-methionine (99%)	.007	---	---	.002	---	.030	---	---	---	---
Salt	.31	.29	.26	.19	.15	.18	.14	.31	.27	.27
Trace min. mic MNTM	.083	.082	.082	.078	.077	.072	.071	.085	.084	.084
Vitamin mix MTG-74	.179	.179	.179	.183	.183	.187	.186	.181	.180	.180
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	16.2	16.7	17.3	17.2	18.3	16.7	17.8	15.1	16.3	16.4
Met. en., kcal/kg	3221	3218	3215	3292	3286	3353	3347	3246	3240	3236

TABLE 3. Body weights of male turkeys in Experiment TG-862.

Treatment *	Age in weeks				
	4	8	12	16	20
	kg				
1. Corn-soy control (normal protein)	0.79ab	3.23a	6.75a	10.33a	13.76a
2. As 1, with 2% feather meal	0.76abc	3.01bcd	6.43bc	10.03ab	13.62ab
3. As 1, with 4% feather meal	0.76abc	3.07abcd	6.53ab	10.11ab	13.48abc
4. As 1, with 7% meat & bone meal	0.80a	3.21ab	6.61ab	10.25a	13.71ab
5. As 4, with 4% feather meal	0.75bc	3.06abcd	6.62ab	10.17ab	13.72a
6. As 4, with 4% blood meal	0.78ab	3.13abc	6.43bc	9.77b	13.31abc
7. As 5 and 6 combined	0.75bc	3.04abcd	6.52ab	10.04ab	13.56abc
8. As 1, lower protein level	0.78ab	3.07abcd	6.40bc	9.98ab	13.23bc
9. As 8, with 4% feather meal	0.70cd	2.87d	6.22c	9.79b	13.09c
10. As 9, using avail. amino acids	0.72d	3.00cd	6.37bc	10.12ab	13.43abc
L. S. D. (P=0.05)	0.04	0.18	0.26	0.36	0.43

a,b,c,d: Duncan's Multiple Range test (P<0.05).

* Levels of feather meal and blood meal were decreased by factors of .9, .8, .7, and .6 in successive feeding periods.

TABLE 4. Cumulative feed/gain for toms in TG-862.

	0-4 Wk	0-8 Wk	0-12 Wk	0-16 Wk	0-20 Wk
1. Corn-soy control	1.70dc	1.97bcd	2.29ab	2.64ab	2.91abc
2. As 1, 2% feather meal	1.82ab	2.05a	2.33a	2.63ab	2.88abcde
3. As 1, 4% feather meal	1.77bc	1.99abcd	2.32a	2.66ab	2.94a
4. As 1, 7% meat+bone meal	1.64d	1.93cde	2.25bc	2.57bc	2.83cde
5. As 4, 4% feather meal	1.73bcd	1.91de	2.21c	2.58bc	2.84bcde
6. AS 4, 4% blood meal	1.62d	1.88e	2.20c	2.57bc	2.81de
7. As 4, 5, and 6 combined	1.71bcd	1.92de	2.21c	2.54c	2.80e
8. As 1, lower protein	1.73bcd	2.02ab	2.34a	2.64ab	2.89abcd
9. AS 8, 4% feather meal	1.89a	2.05a	2.33a	2.67a	2.92ab
10. As 9, avail. a. a.	1.79bc	2.00abc	2.30ab	2.58bc	2.86abcde
L. S. D. (P=0.05)	0.10	0.07	0.06	0.08	0.08

See footnotes in Table 3.

TABLE 5. Composition of diets in Experiment TG-865 (Hens)

Diet	1	2	3	4	5	6	7	8	9	10
0-4 weeks:										
Corn, gr. yellow	43.1	43.1	46.2	43.4	46.4	46.9	48.0	46.7	50.4	53.7
Soybean meal, deh.	46.8	46.8	39.6	41.7	34.6	35.5	30.4	38.0	39.3	31.9
Fish sols. product	2.56	2.56	2.59	2.62	2.65	2.70	2.70	2.65	2.63	2.65
Ferm. res. product	.256	.256	.259	.262	.265	.270	.270	.265	.26	.265
Feather meal, hyd.	---	---	4	---	4	---	4	---	---	4
Meat + bone meal	---	---	---	7	7	7	7	7	---	---
Blood meal, ring	---	---	---	---	---	2.5	2.5	---	---	---
Dicalcium phosphate	2.80	2.80	2.77	1.19	1.16	1.29	1.23	1.24	2.92	2.89
Calcium carbonate	1.61	1.61	1.67	1.09	1.16	1.14	1.19	1.12	1.67	1.73
DL-methionine (99%)	.221	.221	.129	.222	.127	.256	.130	.272	.159	.115
L-lysine HCl (99%)	---	---	.177	---	.177	---	.092	.151	---	.214
Salt	.30	.30	.23	.18	.12	.17	.10	.19	.31	.24
Trace min. mix MNTM	.124	.124	.126	.120	.123	.115	.116	.124	.132	.135
Vitamin mix MTS-74	.256	.256	.259	.262	.265	.267	.268	.265	.263	.265
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	27.4	27.4	27.7	28.7	28.9	28.4	29.3	27.4	24.5	24.7
Met. en., kcal/kg	2852	2852	2878	2917	2943	2972	2983	2943	2921	2946
4-8 weeks:										
Corn, gr. yellow	48.3	51.2	51.2	48.7	51.6	52.0	53.2	51.9	55.2	58.3
Soybean meal, deh.	43.4	36.6	36.6	38.2	31.5	32.4	27.6	34.7	36.3	29.4
Fish sols. product	1.85	1.86	1.86	1.89	1.90	1.92	1.93	1.90	1.89	1.90
Ferm. res. product	.185	.186	.186	.189	.190	.192	.193	.190	.189	.190
Feather meal, hyd.	---	3.7	3.7	---	3.7	---	3.7	---	---	3.7
Meat + bone meal	---	---	---	7	7	7	7	7	---	---
Blood meal, ring	---	---	---	---	---	2.3	2.3	---	---	---
Dicalcium phosphate	2.21	2.17	2.17	.58	.55	.67	.61	.63	2.31	2.28
Calcium carbonate	1.35	1.40	1.40	.83	.88	.87	.91	.85	1.40	1.46
DL-methionine (99%)	.098	.061	.061	.097	.057	.126	.060	.143	.055	.041
L-lysine HCl (99%)	---	.169	.169	---	.168	---	.090	.145	---	.201
Salt	.30	.24	.24	.19	.13	.18	.11	.19	.31	.25
Trace min. mix MNTM	.113	.116	.116	.110	.112	.105	.105	.113	.121	.124
Vitamin mix MTS-74	.205	.207	.207	.210	.211	.213	.214	.212	.209	.211
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	25.8	26.0	26.0	27.0	27.2	26.7	27.5	25.8	23.0	23.2
Met. en., kcal/kg	2927	2951	2951	2994	3018	3046	3056	3020	2992	3016
8-11 weeks:										
Corn, gr. yellow	56.2	58.8	58.8	56.8	59.3	59.8	60.8	59.9	62.3	64.9
Soybean meal, deh.	36.9	31.1	31.1	31.6	25.8	26.4	22.3	28.2	30.7	24.8
Fish sols. product	1.13	1.14	1.14	1.15	1.16	1.17	1.17	1.16	1.15	1.16
Ferm. res. product	.113	.114	.114	.115	.116	.117	.117	.116	.115	.116
Feather meal, hyd.	---	3.1	3.1	---	3.1	---	3.1	---	---	3.1
Meat + bone meal	---	---	---	7	7	7	7	7	---	---
Blood meal, ring	---	---	---	---	---	2	2	---	---	---
Dicalcium phosphate	1.81	1.78	1.78	.17	.15	.24	.19	.21	1.88	1.86
Calcium carbonate	1.17	1.22	1.22	.65	.70	.68	.72	.67	1.22	1.26
DL-methionine (99%)	.021	.023	.023	.017	.019	.041	.020	.060	---	.005

TABLE 5. Composition of diets in Experiment TG-865 (Hens) -- Continued

	1	2	3	4	5	6	7	8	9	10
L-lysine HCl (99%)	---	.145	.145	---	.144	---	.076	.134	---	.170
Salt	.30	.25	.25	.19	.14	.18	.12	.19	.31	.26
Trace min. mix MNTM	.103	.105	.105	.099	.101	.095	.095	.102	.109	.112
Vitamin mix MTG-74	.256	.258	.258	.262	.264	.266	.267	.264	.261	.263
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	22.9	23.1	23.1	24.1	24.2	23.8	24.5	22.9	20.5	20.7
Met. en., kcal/kg	3020	3040	3040	3089	3109	3136	3143	3113	3077	3097
11-14 weeks:										
Corn, gr. yellow	67.0	69.3	69.3	67.8	70.1	70.5	71.4	70.6	71.8	74.2
Soybean meal, deh.	26.8	21.8	21.8	21.3	16.2	16.8	13.2	18.2	22.0	16.8
Fish sols. product	.77	.77	.77	.79	.79	.80	.80	.79	.78	.78
Ferm. res. product	.077	.077	.077	.079	.079	.080	.080	.079	.078	.078
Feather meal, hyd.	---	2.7	2.7	---	2.7	---	2.7	---	---	2.7
Meat + bone meal	---	---	---	7	7	7	7	7	---	---
Blood meal, ring	---	---	---	---	---	1.7	1.7	---	---	---
Dicalcium phosphate	1.58	1.55	1.55	---	---	---	---	---	1.64	1.61
Calcium carbonate	1.09	1.26	1.26	.53	.55	.587	.593	.57	1.12	1.16
DL-methionine (99%)	.034	.021	.021	.030	.017	.051	.018	.068	---	.002
L-lysine HCl (99%)	---	.125	.125	---	.124	---	.067	.120	---	.147
Salt	.31	.26	.26	.19	.15	.18	.14	.19	.31	.27
Trace min. mix MNTM	.093	.095	.095	.089	.091	.085	.085	.092	.098	.099
Vitamin mix MTG-74	.205	.206	.206	.210	.211	.213	.213	.211	.208	.209
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	19.0	19.1	19.1	20.0	20.1	19.7	20.3	19.0	17.1	17.2
Met. en., kcal/kg	3135	3154	3154	3206	3223	3247	3254	3229	3180	3199
14-17 weeks:										
Corn, gr. yellow	74.8	76.9	76.9	75.7	77.8	78.2	79.0	78.3	78.6	80.8
Soybean meal, deh.	19.9	15.3	15.3	14.1	9.6	10.1	6.9	11.4	16.0	11.3
Fish sols. product	.41	.41	.41	.42	.42	.42	.42	.42	.41	.41
Ferm. res. product	.041	.041	.041	.042	.042	.042	.042	.042	.041	.041
Feather meal, hyd.	---	2.4	2.4	---	2.4	---	2.4	---	---	2.4
Meat + bone meal	---	---	---	7	7	7	7	7	---	---
Blood meal, ring	---	---	---	---	---	1.5	1.5	---	---	---
Dicalcium phosphate	1.32	1.29	1.29	---	---	---	---	---	1.36	1.33
Calcium carbonate	.98	1.02	1.02	.27	.29	.32	.32	.30	1.01	1.04
DL-methionine (99%)	.006	.005	.005	.002	---	.020	.001	.036	---	---
L-lysine HCl (99%)	---	.112	.112	---	.112	---	.061	.108	---	.131
Salt	.30	.26	.26	.19	.15	.18	.14	.19	.31	.27
Trace min. mix MNTM	.083	.084	.084	.078	.080	.075	.075	.081	.086	.087
Vitamin mix MTG-74	.179	.180	.180	.183	.184	.185	.186	.185	.182	.182
Animal fat	2	2	2	2	2	2	2	2	2	2
Crude protein, %	16.2	16.3	16.3	17.1	17.2	16.8	17.4	16.2	14.7	14.7
Met. en., kcal/kg	3222	3239	3239	3291	3307	3329	3336	3312	3258	3275

TABLE 6. Body weights of tom turkeys in Experiment TG-865.

Treatment *	Age in weeks				
	4	8	11	14	17
	kg				
1 Control, corn-soy (normal protein)	.84b	3.01ab	4.81a	6.43a	8.15a
2 As 1, 4% feather meal beginning at 4 wks	.86ab	3.03ab	4.83a	6.44a	8.19a
3 As 1, 4% feather meal beginning day of age	.86ab	2.98b	4.83a	6.36a	8.08a
4 As 1, 7% meat and bone meal	.89a	3.09ab	4.89a	6.41a	8.05a
5. As 4, with 4% feather meal	.87ab	3.06ab	4.83a	6.40a	8.14a
6 As 4, with 2.5% blood meal	.87ab	3.08ab	4.90a	6.40a	8.10a
7 As 4, 5, & 6 combined	.87ab	3.11ab	4.90a	6.36a	8.01a
8 As 4, with lower protein	.89a	3.13a	4.88a	6.36a	8.08a
9 As 1, lower level of protein	.77c	2.78c	4.53b	6.10b	7.94a
10 As 9, 4% feather meal - avail. amino acids	.75c	2.64d	4.34c	5.82c	7.64b
L. S. D. (P=0.05)	.04	.13	.18	.21	.23

a,b,c,d: Duncan's Multiple Range test (P<0.05).

* Levels of feather meal and blood meal were decreased by factors of .9, .8, .7, and .6 in successive feeding periods.

TABLE 7. Cumulative feed/gain for hens in TG-865.

	0-4 Wk	0-8 Wk	0-11 Wk	0-14 Wk	0-17 Wk
1. Corn-soy control	1.45bc	1.83b	2.26a	2.57abc	2.91ab
2. As 1, 4% fea at 4 wk	1.40cde	1.82b	2.23ab	2.58ab	2.90ab
3. As 1, 4% fea start	1.42bcd	1.83b	2.25a	2.62a	2.93a
4. As 1, 7% meat	1.34ef	1.78bc	2.16bc	2.51cd	2.80c
5. As 4, 4% fea start	1.36ef	1.78bc	2.19abc	2.53bcd	2.82c
6. As 4, 2.5% blood	1.34f	1.77bc	2.15c	2.49d	2.80c
7. As 4, 5, and 6	1.37def	1.77bc	2.17bc	2.55bcd	2.82c
8. As 4, less protein	1.32f	1.74c	2.16c	2.52bcd	2.81c
9. As 1, lower protein	1.47b	1.79bc	2.18bc	2.53bcd	2.87bc
10. As 9, 4% feather meal, avail. a.a.	1.53a	1.92a	2.25a	2.62a	2.89ab
L. S. D. (P=0.05)	.05	.06	.06	.06	.06

See footnotes in Table 6.