

RELATIONSHIP BETWEEN PROTEIN NUTRITIONAL STATUS AND IMMUNOCOMPETENCE IN NORTHERN BOBWHITE CHICKS

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ABSTRACT.—We investigated the effects of dietary protein quality on the development and functioning of the immune system in four-week-old Northern Bobwhite (*Colinus virginianus*) chicks. Chicks were fed isocaloric diets containing 8, 15, or 33% protein over a three-week period. Significant reductions in the rate of body growth were evident in chicks receiving 8 and 15% protein. Development of the bursa of Fabricius and spleen was significantly depressed in the 8% protein group compared to the other two treatments. Lymphocyte yields from dissociated lymphoid organs of chicks fed 8% protein were substantially reduced compared to birds fed higher levels of protein. *In vitro* lymphoproliferative responses of cultured splenocytes to mitogenic stimulation (concanavalin A, pokeweed mitogen, and *Salmonella typhimurium*), white-blood-cell counts, and *in vivo* measures of humoral immunity did not differ among dietary treatments. Cell-mediated immune function, as measured by an *in vivo* hypersensitivity response to an intradermal injection of a T lymphocyte-dependent mitogen (phytohemagglutinin), was significantly suppressed in the 8% protein group compared to the other two treatments. Several measures of immune-system development and function were significantly correlated with body mass change during the trial. Results indicated that four-week-old Northern Bobwhite chicks fed an 8% protein diet for three weeks may have difficulty expressing a competent immune response to pathogenic challenge in the wild. Received 30 January 1992, accepted 25 November 1992.

NORTHERN BOBWHITE (*Colinus virginianus*) populations in the central Great Plains fluctuate widely and unpredictably within and among years (Stanford 1972, Brennan 1991). Net productivity during summer is highly dependent upon nesting success and chick survival (Roseberry and Klimstra 1972, Cantu and Everett 1982). Chick mortality is often high during the first few weeks of development and appears to be related to range condition (Cantu and Everett 1982). Causes of chick mortality are difficult to assess in the wild, but nutrition has been suggested as an important consideration (Hurst 1972). Juvenile birds have a high requirement for dietary protein (Nestler et al. 1942), which necessitates the consumption of invertebrates to meet this high demand (Hurst 1972).

The physiological consequences of moderate to low dietary protein deficiencies in juvenile Northern Bobwhite are not completely known, but reduced growth and development has been documented under controlled conditions (Nestler et al. 1942). Biomedical research has demonstrated a relationship between nutrition and immunocompetence in humans and laboratory animals (reviewed by Chandra and Newberne 1977, Gershwin et al. 1985). Similar relation-

ships undoubtedly occur in upland game birds, but have not been adequately studied to determine the sensitivity of immune-system development to early protein malnutrition. Several studies on domestic poultry have demonstrated alterations in immune-organ development and functioning of both the humoral and cell-mediated arms of the immune system in response to acute and chronic malnutrition (Glick et al. 1981, 1983, Willis and Baker 1981, Klasing 1988).

Our objective was to explore the effects of dietary protein quality on the development of immunocompetence in juvenile Northern Bobwhite under controlled conditions. Lymphoid-organ development, lymphocyte populations, *in vitro* lymphoproliferative responses, and *in vivo* measures of humoral and cell-mediated immunity were evaluated in four-week-old chicks subjected to a three-week feeding trial.

MATERIALS AND METHODS

Experimental subjects.—Juvenile Northern Bobwhite exactly four weeks old were obtained from the El Reno Game Farm, Canadian County, Oklahoma, which is operated by the Oklahoma Department of Wildlife Conservation for the captive propagation of upland

TABLE 1. Ingredient composition of isocaloric 8, 15, and 33% protein diets fed to juvenile Northern Bobwhites from four to seven weeks of age.

Ingredient	Percent air-dry basis		
	8% diet	15% diet	33% diet
Starch	53.31	45.60	30.00
Ground corn	21.76	21.35	20.94
Iso-soy ^a	7.56	16.16	32.66
Animal fat	12.09	11.89	11.63
Dicalcium phosphate ^b	3.32	2.96	2.61
Vitamin mix ^c	0.45	0.44	0.44
Methionine, 99%	0.45	0.44	0.44
Salt	0.45	0.44	0.44
Limestone, 38% Ca	0.45	0.57	0.68
Trace mineral mix ^d	0.15	0.15	0.15

^a Soybean meal protein, 84.5% crude protein content (Protein Technologies International).

^b 18.5% P, 22% Ca, 0.18% F1 (Pitman-Moore, Inc.).

^c Per pound of diet: 1,800,000 IU vitamin A, 500,000 IU vitamin D₃, 6,000 IU vitamin E, 3.6 mg vitamin B₁₂, 1,200 mg riboflavin, 8,000 mg niacin, 2,000 mg d-pantothenic acid, 90,800 mg choline, 330 mg menadione, 200 mg folic acid, 720 mg pyridoxine, 360 mg thiamine, 20 mg d-biotin (Hoffman-LaRoch, Inc.).

^d 15.00% Ca, 10.00% Zn, 12.00% Mn, 7.50% Fe, 1.00% Cu, 0.25% I (J. M. Huber Corp.).

game birds. We used chicks four weeks old because of their large rate of growth, increased dietary requirement for protein, and body size. Chicks younger in age were difficult to use for selected *in vivo* immune-response assays. Thirty-six juveniles were weighed and randomly assigned to one of three experimental dietary-protein treatments (8, 15, and 33% protein) for a three-week trial from 1 to 22 April 1991. Birds were raised under a natural photoperiod in a battery brooder containing vertical decks (4 × 4 × 2 m) located in a well-ventilated facility approved by the Institutional Laboratory Animal Resources Committee at Oklahoma State University. Temperature in the housing facility was maintained within the range of 19 to 28°C (\bar{x} = 23.3) using heat lamps and regulating ventilation. Water and experimental diets were provided *ad libitum* throughout the trial. Experimental protein diets were made isocaloric by varying the concentration of starch (Table 1). Daily food consumption was measured to insure palatability by offering a known quantity of food and weighing uneaten portions the following day (correcting for spillage). Body mass was determined at weekly intervals during the three-week trial.

Birds were returned to the laboratory after the trial, anesthetized with 5 mg ketamine hydrochloride, and exsanguinated via the jugular vein. Uncoagulated blood samples were collected in EDTA (K₃) supplemented vacuum tubes for hematological analyses. White-blood-cell (WBC) counts were made using a hemacytometer and Natt-Herrick's solution (Natt and Herrick 1952), and hematocrits determined using the microcapillary-tube method (Gee et al. 1981). Birds were necropsied, and spleen and bursa of Fabricius

aseptically removed and weighed. Lymphoid-organ cellularity was determined by gently dissociating spleen and bursa, and counting viable lymphocytes using a hemacytometer and a trypan-blue exclusion technique.

Cell-mediated immunity.—A phytohemagglutinin-P (PHA) injection assay (Cheng and Lamont 1988) was used to evaluate *in vivo* T-cell-mediated immune response of Northern Bobwhite chicks during the last day of the trial. Birds were injected intradermally in the right wing web with 0.5 mg of PHA (Sigma Chemical Co., St. Louis, Missouri) in 0.1 ml of phosphate buffered saline (PBS) after marking the injection site. The opposite wing web (control) was injected with 0.1 ml of PBS. The thickness of each wing web was measured (to nearest 0.001 in [ca. 0.02 mm]) at the injection site just prior to and 24 h after challenge with a pressure-sensitive micrometer. Wing-web swelling was calculated as the difference between the thickness of the wing web prior to and after (24 h) injection of PHA. The cell-mediated immune response (wing-web index) was calculated as the difference in wing-web swelling between the PHA-injected and control sites. The degree of erythema at the site of induration was subjectively assigned a numerical score as described by Kramer and Good (1978): (0) none, (1) slight, (2) moderate, (3) intense.

Humoral immunity.—*In vivo* humoral immune response was assessed by measuring primary antibody responses to a single injection with heterologous erythrocytes (Tsiagbe et al. 1987). Six days prior to termination of the trial, birds were injected intraperitoneally with 0.5 ml of a 5.0% sheep red-blood-cell (SRBC) suspension (vol/vol) in PBS. Blood samples for serum were obtained from the jugular vein at termination (day 6 postinjection) to evaluate primary hemagglutinating antibody responses to SRBC inoculation. Hemagglutinating antibody titers were measured by a microhemagglutination assay (Wegmann and Smithies 1966). Briefly, serial two-fold dilutions of heat-inactivated serum (56°C for 30 min) in PBS were mixed with an equal volume of a 1% SRBC solution (in PBS) and incubated at 40°C for 1 h. Titers were expressed as the log₂ of the reciprocal of the highest dilution of serum showing positive hemagglutination.

Lymphoproliferative responsiveness.—Aseptically removed spleens were dissociated in a sterile glass-on-glass tissue homogenizer containing 5 ml supplemented RPMI 1640 medium (RPMI-S) consisting of 1.025% L-glutamine (200 mM), 1.0% Na pyruvate (100 mM), 1.0% nonessential amino acids (10 mM), 1.0% penicillin (10,000 U/ml)-streptomycin (10 mg/ml) solution, 0.1% 2-mercaptoethanol (2×10^{-2} M, 1:1,000 dilution in sterile PBS), heparin (10 u/ml), and 5.0% normal chicken serum (all reagents, unless stated otherwise, obtained from Sigma Chemical Co., St. Louis, Missouri). Cells were centrifuged for 7 min at 10°C and $275 \times g$, supernatant decanted, and pellet resus-

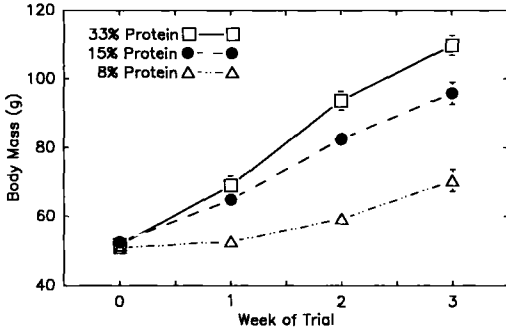


Fig. 1. Body-mass gain ($\bar{x} \pm SE$) of Northern Bobwhite chicks fed formulated isocaloric rations containing 8, 15, or 33% protein from four to seven weeks of age.

pended in 5 ml RPMI-S (this wash step was repeated twice). Viable-cell counts were performed with a hemacytometer using trypan-blue exclusion; mononuclear cell viability was consistently more than 90%.

Lymphocyte proliferation of T and B cells in response to polyclonal mitogenic stimulation was assessed *in vitro* using the plant lectins concanavalin A (Con A, *Canavalia ensiformis*) and pokeweed mitogen (PWM, *Phytolacca americana*), and a protein extract from cell walls of *Salmonella typhimurium* (STM from RIBI Immunochem Research, Hamilton, Montana). The spleen-cell suspension was adjusted to a final concentration of 500,000 cells/90 μ l in RPMI-S. Four concentrations of each mitogen (Con A at 2.5, 5, 10, and 20 μ g/ml of culture; PWM at 1.25, 2.50, 5.0, and 10.0 μ g/ml of culture; STM of 1.25, 2.5, 5, and 10 μ g/ml culture) were added (10 μ l volumes) to duplicate 90- μ l aliquots of the final cell suspension in 96-well, flat-bottom microtiter plates. Unstimulated control cultures were used as blanks.

Cell cultures were incubated for 72 h at 40.5°C in a humidified water-jacketed incubator with 5% CO₂-95% air. Lymphocyte proliferation was assessed by cellular reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) using the procedure described by Mosmann (1983). Endpoints (stimulation index) were recorded as ratios of absorbances at 570/630 nm of stimulated cultures to unstimulated controls.

Statistical analyses.—Differences in lymphoid organ morphology and cellularity, hematology, hemagglutination titers, PHA wing-web indices, and lymphoproliferative stimulation indices among dietary protein treatments were tested by ANOVA. The *F*-max test was used to test for homogeneity of sample variances for all dependent variables prior to ANOVA (Sokal and Rohlf 1969). Heteroscedasticity characterized all variables related to the bursa (mass and cellularity), so data were log-transformed prior to further statistical analyses. We used the least significant

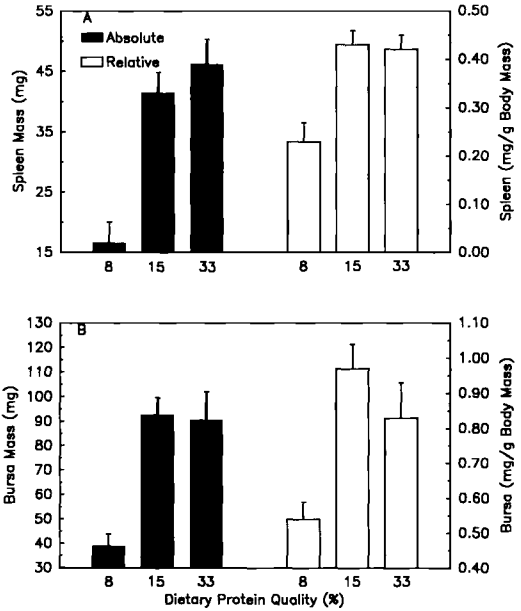


Fig. 2. Mean \pm SE absolute (mg) and relative (mg/g body mass) masses of lymphoid organs of seven-week-old Northern Bobwhite chicks fed isocaloric diets containing 8, 15, or 33% protein over a three-week period.

difference test to isolate differences among means ($P < 0.05$). Relationships among immune-system parameters and body-mass variables were evaluated using Pearson correlation analysis.

RESULTS

Two juvenile bobwhites (one from 8 and 33% groups) died during the experimental trial due to unknown causes and were deleted from all analyses. Initial body masses averaged $51.8 \pm SE$ of 0.8 g and did not differ ($P = 0.680$) among treatment groups. All three isocaloric protein diets were highly palatable. Average feed consumption for the 33, 15, and 8% protein treatment groups were 11.0 ± 0.4 , 10.0 ± 0.4 , and 9.2 ± 0.3 g·bird⁻¹·day⁻¹ during the trial and reflected differences in average body mass. Body growth rate as measured by percent change in body mass during the trial differed significantly ($P = 0.001$) among all three treatment groups (Fig. 1). All chicks continued to grow during the experimental trial; body-mass change averaged $38.7 \pm 5.3\%$, $82.6 \pm 4.9\%$, and $112.2 \pm 4.0\%$ in the 8, 15, and 33% protein groups, respectively.

Morphology and cellularity.—Hematological

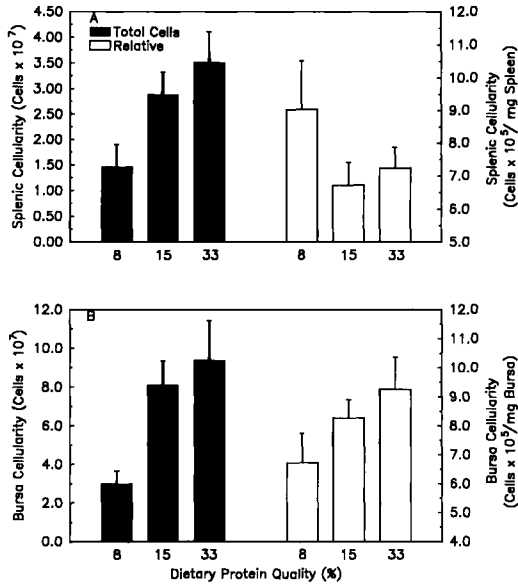


Fig. 3. Mean \pm SE total (per organ) and relative (per mg organ) lymphocyte yields from spleen and bursa of Fabricius in seven-week-old Northern Bobwhite chicks fed isocaloric diets containing 8, 15, or 33% protein over a three-week period.

parameters were not influenced ($P > 0.400$) by dietary protein quality. Overall white-blood-cell counts and hematocrits averaged $22.34 \times 10^3 \pm 1.59 \times 10^3/\text{mm}^3$ and $35.9 \pm 0.6\%$, respectively.

Absolute and relative (mg/g body mass) masses of spleen ($P = 0.001$ and 0.001) and bursa ($P = 0.001$ and 0.001 , respectively) were significantly influenced by dietary protein treatment (Fig. 2). Both the 15 and 33% diet protein groups had absolute and relative masses greater than chicks in the 8% diet group; differences between the 15 and 33% dietary protein groups were not significant. Total lymphocyte yields from dissociated spleen ($P = 0.021$) and bursa ($P = 0.002$) were influenced by dietary protein (Fig. 3). Yields were lower in the 8% protein group (58% reduction in cell yield for spleen and 68% reduction for bursa compared to 33% group) than the other two treatments; there was no difference between 15 and 33% dietary protein groups. Lymphocyte yields from spleen ($P = 0.244$) and bursa ($P = 0.165$), expressed on a relative basis (cells/mg tissue), showed no relationship to dietary protein content (Fig. 3).

In vivo immune responses.—There was no difference ($P = 0.554$) among treatments in serum hemagglutinating antibody titers of SRBC-im-

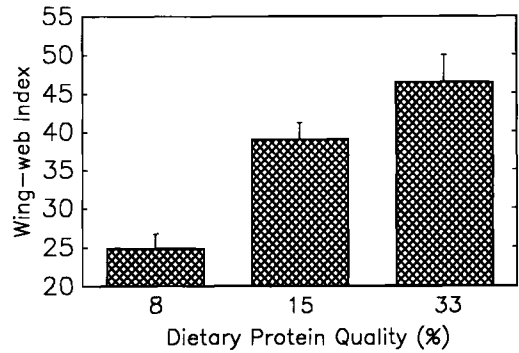


Fig. 4. Cell-mediated immune responses to an intradermal injection of phytohemagglutinin in seven-week-old Northern Bobwhite chicks fed isocaloric diets containing 8, 15, or 33% protein over a three-week period. Responses expressed as wing-web index ($\bar{x} \pm \text{SE}$), which reflected degree of swelling in skin 24-h postinjection.

munized chicks. All chicks expressed a primary antibody response to SRBC immunization. Hemagglutinating antibody titers (\log_2) varied from 2 to 8 with an overall mean of 4.58 ± 0.33 .

In vivo cell-mediated immune response as measured by PHA stimulation (wing-web index) was suppressed ($P = 0.001$) in the 8% dietary protein group compared to other treatments (Fig. 4). There was no difference ($P > 0.05$) in the mean wing-web index between 15 and 33% dietary protein treatments. Birds on both the 8% (1.5 ± 0.28) and 15% (2.27 ± 0.20) protein diets displayed smaller skin reactions that were less erythematous than bobwhite chicks on the 33% protein group (2.64 ± 0.16); three chicks on the 8% diet displayed no erythema to slight erythema, while all chicks in the 33% group displayed moderate to intense reactions. The magnitude of the PHA response was significantly correlated with body-mass change, final body mass, and development of the spleen and bursa (Table 1).

Lymphoproliferation.—Lymphocyte yields from dissociated spleen preparations of chicks from low protein diet groups were frequently inadequate to test lymphoproliferative responsiveness at all mitogen dosages (Table 3). The ability of cultured splenocytes of bobwhite chicks to proliferate *in vitro* when stimulated with the mitogens Con A, PWM, and STM did not appear to be fully developed. Stimulation indices for Con A showed a clear dose response from 2.5 to 20.0 $\mu\text{g}/\text{ml}$, but a similar response was not

TABLE 2. Pearson correlation coefficients for significant relationships between selected measures of immune-system function, body mass, and immune-organ development in juvenile Northern Bobwhites fed an 8, 15, or 33% protein diet for three weeks.

Immune parameter	Change in body mass	Final body mass	Splenic cellularity	PHA response
Spleen mass	0.66***	0.75***	0.86***	0.76***
Splenic cellularity	0.51**	0.63***	—	0.63***
Bursa mass	0.56***	0.62***	0.53**	0.71***
Bursa cellularity	0.45**	0.54***	0.50**	0.67***
PHA skin response	0.71***	0.71***	—	—
Lymphoproliferation ^a				
Con A, 2.5 µg/ml	0.35*	0.36*	0.48**	—
5.0	0.38*	0.46*	0.61***	0.43**
10.0	—	0.54**	0.62***	0.42*
20.0	—	—	0.73***	0.53*
STM, 1.25 µg/ml	—	0.44*	0.46*	—
2.50	—	—	0.51**	—
5.0	—	—	0.50**	—
10.0	—	—	0.61***	—
PWM, 0.156 µg/ml	0.46*	0.54**	0.52*	0.54*
0.313	—	—	—	0.44*

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

^a Splenocytes cultured with varying dosages of mitogens concanavalin A (Con A), pokeweed mitogen (PWM), or *Salmonella typhimurium* (STM).

evident for PWM and STM. Overall, lymphoproliferative responses were negligible for PWM and low to moderate for Con A and STM.

Dietary protein had no ($P > 0.10$) influence on stimulation indices of cultured splenocytes for Con A, PWM, and STM at the dosages used. However, correlation analysis revealed significant relationships between body-mass change or final body mass and stimulation indices for the three mitogens at several concentrations (Table 2). Lymphoproliferative responses to

several mitogens and dosages were also correlated with bobwhite chick responsiveness to *in vivo* PHA challenge and splenic cellularity.

DISCUSSION

Northern Bobwhite chicks require a substantial intake of protein to sustain optimum growth during the first few weeks posthatching (Nestler et al. 1942). Several studies recommend at least a 28% concentration of protein in the diet

TABLE 3. Lymphoproliferative responses^a of cultured splenocytes stimulated with various dosages of concanavalin A (Con A), pokeweed mitogen (PWM), and *Salmonella typhimurium* (STM). Juvenile Northern Bobwhites were fed an 8, 15, or 33% protein ration for three weeks. Presented as $\bar{x} \pm SE$ (n).

Dosage (µg/ml)	8%	15%	33%	Overall
Con A				
2.5	1.124 ± 0.028 (10)	1.151 ± 0.042 (12)	1.214 ± 0.037 (11)	1.164 ± 0.022 (33)
5.0	1.153 ± 0.025 (11)	1.242 ± 0.067 (12)	1.337 ± 0.068 (11)	1.244 ± 0.034 (34)
10.0	1.212 ± 0.075 (4)	1.304 ± 0.081 (11)	1.447 ± 0.075 (11)	1.351 ± 0.050 (26)
20.0	1.439 ± 0.152 (2)	1.378 ± 0.091 (8)	1.504 ± 0.102 (10)	1.447 ± 0.063 (20)
PWM				
0.156	1.003 ± 0.025 (3)	1.031 ± 0.023 (8)	1.059 ± 0.034 (10)	1.040 ± 0.018 (21)
0.313	0.993 ± 0.059 (4)	0.987 ± 0.026 (11)	1.028 ± 0.037 (11)	1.006 ± 0.020 (26)
0.625	1.004 ± 0.057 (6)	0.975 ± 0.019 (12)	0.997 ± 0.049 (11)	0.989 ± 0.022 (29)
1.250	1.008 ± 0.073 (5)	0.956 ± 0.030 (12)	0.921 ± 0.046 (11)	0.951 ± 0.026 (28)
STM				
1.25	1.282 ± 0.056 (3)	1.185 ± 0.040 (8)	1.301 ± 0.047 (10)	1.253 ± 0.030 (21)
2.50	1.242 ± 0.067 (4)	1.181 ± 0.054 (11)	1.268 ± 0.055 (11)	1.227 ± 0.034 (26)
5.0	1.206 ± 0.039 (11)	1.186 ± 0.040 (12)	1.258 ± 0.062 (11)	1.216 ± 0.027 (34)
10.0	1.206 ± 0.056 (9)	1.222 ± 0.059 (12)	1.281 ± 0.084 (11)	1.238 ± 0.038 (32)

^a Lymphoproliferative response measured as ratio of absorbances of stimulated/unstimulated culture wells.

to support optimum growth and survival to six weeks of age (Nestler et al. 1942, Baldini et al. 1950, Andrews et al. 1973). Protein requirements for bobwhite chicks appear to drop to about 20% from six to nine weeks of age (Andrews et al. 1973). Inability of chicks to acquire adequate-quality rations during this period of intense development will result in significant depressions in growth and development as indicated by the masses of bobwhite chicks fed a 15 or 8% protein diet in our study.

Severe atrophy of major lymphoid organs occurs in animals subjected to protein malnutrition during early development (Bell et al. 1976). Lymphoid organs are centers of intense cell division and proliferation that can be severely restricted when nutrients are not available in protein malnutrition. Similarly, our study demonstrated that growth and development of the bursa and spleen are particularly susceptible to dietary protein deficiencies from four to seven weeks of age in Northern Bobwhite. Spleen and bursa masses reflected about 178 and 132% greater growth by seven weeks of age in the high-protein-diet group compared to the 8% group. These results suggest that lymphoid organs of bobwhites may be more sensitive to early protein malnutrition than those of chickens. Glick et al. (1981) observed significant reductions in growth of the bursa but no depression in spleen development in five-week-old poultry chicks fed one-third of the protein in a basal diet since hatching.

Organ cellularity was determined to assess the effect of this depressed development on the ability of these primary (bursa) and secondary (spleen) lymphoid organs to maintain and generate adequate numbers of viable lymphocytes. The reduction in lymphoid organ cellularity was proportional to the degree of growth as indicated by similar relative cell yields (cells/mg tissue) among dietary treatments. Marsh et al. (1986) observed a similar proportional relationship between mass and cellularity in one- to five-week-old poultry chicks fed selenium- and vitamin-E-deficient diets.

Considering the importance of the bursa, and to some degree the spleen, in B lymphocyte development and creation of an antibody repertoire (Toivanen et al. 1987), reductions in bursa growth due to protein malnutrition might be expected to adversely influence humoral immune responses. However, bobwhite chicks on low-protein diets were fully capable of pro-

ducing an *in vivo* antibody response to SRBC immunization, a T-lymphocyte-dependent antigen, suggesting that diet did not negatively influence the immunocompetence of B lymphocytes. This may be due in part to the observation that primary immune response to SRBC is largely an IgM response, which is not as dependent on a fully developed bursa as an IgG response (Toivanen et al. 1987). Poultry chicks subjected to severe protein restriction exhibit a more dramatic depression in the secondary IgG response than the primary response to SRBC immunization (Glick et al. 1981). Lesions in the primary response to SRBC appear to be dose dependent as well (Glick et al. 1981).

The PHA intradermal reaction, a T-lymphocyte-dependent response, has been well researched and has been shown to be a reliable indicator of *in vivo* cellular immunity in poultry (Goto et al. 1978, McCorkle et al. 1980). The skin response reflects a complex series of physiological events such as mitogen-receptor and lymphocyte-macrophage interactions, release of chemical mediators, cellular proliferation, and changes in vascularity (Chandra and Newberne 1977). Histologically, PHA is strongly mitogenic to T lymphocytes, and intradermal injections elicit macrophage infiltration and dense perivascular accumulations of lymphocytes 24 h postinjection in chickens (Goto et al. 1978, McCorkle et al. 1980). The increased infiltration by basophils and eosinophils 24 h postinjection has been described as a cutaneous basophil hypersensitivity response (Stadecker et al. 1977). Wing webs of bobwhite chicks injected with PHA were characterized by varying degrees of erythema and induration, which was related to dietary protein treatment (data not shown) and correlated with the wing-web index.

Our results are in general agreement with previous studies demonstrating impairments in cell-mediated, T-lymphocyte-dependent immune functions in cases of protein malnutrition (Gershwin et al. 1985). Depressed *in vivo* PHA responsiveness has been observed in poultry chicks subjected to amino-acid-deficient diets (Tsiagbe et al. 1987). A similar study by Glick et al. (1983) noted significant reductions in delayed hypersensitivity to intradermal injections of human gamma globulin in protein-deficient poultry chicks. The exact physiological mechanisms by which protein deficiencies elicit alterations in T-lymphocyte-dependent immune function is difficult to ascertain and probably

involves a variety of systems. Inhibition of selected aspects of macrophage function may contribute to overall alterations in immune function since they are involved in antigen presentation, mitogen-induced proliferation, interleukin-1 production, as well as many other crucial roles (Wan et al. 1989). Our observations of variable *in vitro* lymphoproliferative responsiveness to polyclonal activation, which involves macrophage function, but significantly reduced cellularity in primary and secondary lymphoid organs suggest that reduced numbers of immunocompetent cells were partly responsible for depressed *in vivo* responses to PHA. However, it remains possible that selected T lymphocyte subpopulations may have been more sensitive to protein restriction in the diet.

Northern Bobwhite chicks reside in a complex antigenic environment where a completely competent immune system is undoubtedly a necessity for a high probability of survival to adulthood. Any significant compromise in immunocompetence could be expected to lead to higher morbidity and mortality (either directly from disease or indirectly through predation) during early development and subsequent reductions in overall recruitment into the fall population. Studies with laboratory rodents have demonstrated a strong relationship between resistance to many bacterial and parasitic infections and protein nutritional status. This study reinforces previous research results demonstrating that bobwhite chicks have a high protein requirement for normal growth and deficiencies in the diet can lead to severe atrophy or suppressed development in both primary and secondary lymphoid organs. Evidence for suppressed cell-mediated immune function was also strong for bobwhite chicks subjected to early protein malnutrition. It seems reasonable to assume that protein-restricted bobwhite chicks would have difficulty mounting a competent immune response to pathogenic challenges in the wild.

No study has conclusively linked a decline in bobwhite chick survival to availability of high-protein food stuffs (insects) in the habitat during the period of rapid development. However, relationships between weather (rainfall) and recruitment or population density have been explored and suggest a nutritional link (Lehmann 1984, Guthery et al. 1988). The model we describe could be an important mechanism whereby weather, which regulates primary

production and insect population dynamics, influences juvenile survival in Northern Bobwhite populations.

Additionally, several measures of immunocompetence that we examined could also be used as indices of nutritional status in wild populations of Northern Bobwhites. Immunological tests are routinely used as functional indices of protein-calorie malnutrition in humans (Neumann et al. 1975, Gibson 1990). For juvenile Northern Bobwhites, mass and cellularity of primary and secondary lymphoid organs and the *in vivo* PHA skin response assay are easy to perform and sensitive to dietary-protein quality.

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