Maternal antibody transfer to broiler progeny varies among strains and is affected by grain source and cage density[1]

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ABSTRACT Two experiments were conducted to examine the effects of broiler breeder dietary grain source and cage density on maternal antibody (MatAb) transfer to progeny in 2 genetic strains (A and B). Broiler breeders were assigned to 16 litter floor pens and fed either corn- or wheat-based diets. Breeders were administered 4 live vaccines against Newcastle disease virus (NDV). At 23 wk of age, pullets and cocks, which reflected the full BW distribution from each treatment, were moved to a cage breeder house and placed at 1 or 2 hens/cage. Breeders were artificially inseminated at 44 wk (experiment 1) and 52 wk of age (experiment 2). Eggs were collected for 8 d, incubated, and placed in individual pedigree bags at d 19 of incubation. Blood samples from 5 chicks per treatment combination were collected at hatch in both experiments. Spleen and bursa were collected from the same chicks for histomorphometry analyses in experiment 2. In the second experiment, 12 chicks per treatment were placed in cages. Progeny were provided diets based on the same grain (corn or wheat) as their parents. Serum samples were collected at 5, 9, and 13 d of age and analyzed for anti-NDV MatAb. Data were analyzed as a 2 × 2 × 2 factorial design considering strain, dietary grain source, and cage density as main factors. Interaction effects were observed in breeders and progeny. Experiment 1 showed that strain A chicks had lower levels of MatAb when hens were housed at 2 hens/cage rather than 1 hen/cage. The MatAb levels of strain B chickens were not affected by cage density in either experiment. Experiment 2 demonstrated similar effects of cage density on MatAb levels and the area of bursa follicles for both strains. Progeny of breeders fed corn-based diets had smaller spleen white pulp only when hens were housed at 2 hens/cage compared with 1 hen/cage. The results of these experiments suggest that breeder strain and cage-density conditions affected MatAb transfer to progeny and embryo development of spleen and bursa.

Key words: breeder nutrition, broiler progeny, genetic strain, lymphoid tissue

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INTRODUCTION

In broiler production, having high-quality 1-d-old chicks is crucial to obtaining optimal broiler performance (Decuypere et al., 2001). Chick quality is determined by the genetic line of the breeders, breeder age, breeder nutrition, egg weight, egg storage, and incubation conditions (Decuypere et al., 2001; Kidd, 2003; Tona et al., 2003). Yassin et al. (2009) observed that first week mortality of broilers was related to the nutrition of broiler breeders and genetics, among other factors. Maternal transfer of antibodies via the egg yolk has been shown to be important for offspring survivability and growth rate, and functions by passively protecting hatchlings from common pathogens before their endogenous immune system matures (Nordskog and Pevzner, 1977; Grindstaff et al., 2003; Hasselquist and Nilson, 2009).

Chicken offspring have been reported to be capable of producing their own antibodies by 5-d post-hatch, but they typically depend on passive immunity from maternal immunoglobulin Y for the first 13 d post-hatch (Rose and Orlans, 1981; Apanius, 1998). Nevertheless, genetic selection can affect the quantity and quality of maternal antibody (**MatAb**) transfer, as well as how long these antibodies can be maintained in the progeny (Bumstead et al., 1993; Boa-Amponsem et al., 1997; Grindstaff et al., 2003). Selection for higher humoral immune responsiveness in hens and greater MatAb transmission to offspring may also have correlated effects that decrease cell-mediated and innate immunity in the progeny (Ubosi et al., 1985).

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Several literature reports have indicated that breeder nutrition affected antibody production and transfer to chicks (Grindstaff et al., 2003; Kidd, 2003; Hasselquist and Nilson, 2009). Maternal dietary vitamin E restriction decreased antibody transmission into the eggs of chickens (Jackson et al., 1978). Further, the dietary ratio of linoleic acid to α-linoleic acid in broiler breeder feed reduced MatAb transfer to chicks, but it did not affect hen humoral response (Wang et al., 2004). These observations indicated that dietary fatty acid composition may have influenced the binding activity of the immunoglobulin Y receptor on the yolk sac membrane, or that fatty acids affected the half-life of antibodies in the embryo (Wang et al., 2004). Maternal carotenoid nutrition has also been reported to be necessary for the deposition of carotenoids in the lymphoid tissues and skin of the progeny and their immune status (Koutsos et al., 2003). In fact, almost all nutrients in the diet have been shown to play a fundamental role in sustaining an optimal immune response (Kogut and Klasing, 2009). Deficient or excessive nutrient intakes can have negative consequences on immune status and susceptibility to a variety of pathogens (Kogut and Klasing, 2009), and some nutrients may be required for immunity at levels greater than those required for growth or reproduction (Rosales, 1994).

Corn and wheat are the main cereal grain ingredients used in poultry diets worldwide. The usage of both grains is concentrated in specific geographic regions of the world. Wheat is mostly used in Europe, Australia, and Canada (Bird, 1997; International Grains Council, 2004), whereas corn is widely used in the United States, Brazil, and other large poultry-producing countries. Grain source for breeder diets has been reported to affect offspring growth and development (Surai and Sparks, 2001; Eusebio-Balcazar, 2010). Diets formulated with these ingredients may vary in nonstarch polysaccharides, macro and trace minerals, vitamins, phytate, intrinsic phytase, carotenoids, and fatty acids. These nutrient and nonnutrient components may affect avian antioxidant systems and immunology according to several research reports (Surai and Sparks, 2001; Koutsos et al., 2003; Kogut and Klasing, 2009). Therefore, it was important to investigate the possible effects on MatAb transfer when feeding diets containing these 2 types of cereals to broiler breeders.

In broiler breeders, quantitative feed restriction has long been practiced to control BW gain because rapid growth and excessive breast muscle development result in reproductive problems. Under commercial conditions, breeder flocks have been managed to guarantee that the limited amount of feed is uniformly distributed, which can be achieved by providing adequate feeder space to allow for the majority of the pullets to have access to feed at the same time (Leksrisompong, 2010). The occurrence of aggression in a flock when birds have to compete for limited feeder space or when attempts to feed have been frustrated can be stressful (Rosales, 1994). Stress is well recognized to have negative effects on the immune response (Minozzi et al., 2008), including reduced natural killer cell activity, lymphocyte population, lymphocyte proliferation, and antibody production (Webster Marketon and Glaser, 2008). In contrast, several studies have shown that cage density has no significant effect on hemagglutinin titers to sheep red blood cell antigen, percentage of heterophils, lymphocytes, the heterophil to lymphocyte ratio (Patterson and Siegel, 1998), or Newcastle disease virus (**NDV**) antibodies (Tactacan et al., 2009). Several studies (Lochmiller et al., 1993; Patterson and Siegel, 1998; Alonso-Alvarez and Tella, 2001; Cheema et al., 2003; Tactacan et al., 2009) have reported the effects of nutrient availability on cell-mediated immunity within a generation, but very few have investigated whether limited feed availability influenced MatAb transmission to the progeny (Grindstaff et al., 2005). These studies were conducted to examine the effects of broiler breeder dietary grain source and single or double cage density on MatAb transfer to broiler chicken progeny in 2 genetic strains.

MATERIALS AND METHODS

The experiments reported herein were conducted according to the guidelines of the Institutional Animal Care and Use Committee at North Carolina State University (Raleigh). All husbandry practices and euthanasia were carried out with full consideration of animal welfare.

Broiler Breeder Treatment

Two commercial broiler strains indicated here as A and B were selected for this evaluation, taking into consideration that these are the most widely used in the US poultry industry. Strain A has been selected for fast early growth rate and better feed conversion and strain B has been selected for breast meat yield after 7 wk of age. Strain B has higher eggshell conductance than strain A. One-day-old broiler breeder females of both strains were housed together in 16 litter floor pens and fed either corn- or wheat-based diets during rearing and production, following a 4/3-d feed restriction program from 3 to 22 wk, which means that the daily feed allocation was multiplied by 7, divided by 4, and fed Monday, Wednesday, Friday, and Saturday each week. After photostimulation, all breeders were allocated to a daily feeding program. Pullets and cocks were fed starter diets until 4 wk of age that contained 2,900 kcal/kg of ME, 19.21% CP, 1.0% Ca, 0.5% nonphytate phosphorus, 1.05% lysine, and 0.78% TSAA. The grower diets were fed from 5 to 22 wk and contained 2,750 kcal/kg of ME, 15% CP, 0.95% Ca, 0.45% nonphytate phosphorus, 0.75% lysine, and 0.60% TSAA. A pre-lay diet was fed from 18 to 22 wk of age only to breeders that were provided wheat-based diets, which was the same as the grower diet, except with Ca increased to 1.5%. All diets

Table 1. Calculated composition of broiler breeder and broiler experimental diets

	Breeder diet (%)		Broiler diet (%)	
Item	Corn	Wheat	Corn	Wheat
Ingredient				
Corn	60.77	15.01	55.75	20.00
Soybean meal, 48%	19.08	12.25	29.29	24.44
Wheat bran	8.00	—	—	—
Soft wheat grain	—	59.87	—	37.43
Poultry fat	2.30	2.63	2.80	4.05
Distillers dried grains with solubles	—	—	4.75	5.00
Poultry by-product meal	—	—	2.72	4.50
Dicalcium phosphate, 18.5%	2.09	2.10	1.67	1.57
Limestone (fine)	6.41	6.46	1.13	1.05
Sodium bicarbonate	0.06	0.12	0.18	0.21
Salt	0.46	0.37	0.36	0.30
Mineral premix[1]	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.10	0.10	0.10	0.10
Selenium premix, 0.02%[3]	0.10	0.10	—	—
Choline chloride, 60%	0.20	0.20	0.20	0.20
Biotin	0.05	0.05	—	—
Lysine, 98.5%	—	0.21	0.26	0.34
Methionine, 99%	0.10	0.15	0.29	0.29
L-Threonine	0.03	0.13	0.18	0.20
Coccidiostat[3]	0.05	0.05	0.05	0.05
BMD[4]	—	—	0.05	0.05
Phytase (Ronozyme P CT)[5]	—	—	0.02	0.02
Total	100.00	100.00	100.00	100.00
Calculated analyses				
ME (kcal/kg)	2,850	2,850	3,050	3,050
CP	15.60	15.60	22.70	22.70
Calcium	3.00	3.00	0.98	0.98
Nonphytate phosphorus	0.45	0.45	0.49	0.49
Lysine[6]	0.81	0.81	1.25	1.25
Total sulfur amino acids[6]	0.65	0.65	0.92	0.92
Threonine[6]	0.57	0.57	0.85	0.85
Tryptophan[6]	0.17	0.18	0.21	0.24
Isoleucine[6]	0.65	0.60	0.84	0.80
Valine[6]	0.78	0.69	0.98	0.95
Arginine[6]	1.01	0.91	1.33	1.33
Sodium[6]	0.22	0.22	0.23	0.23
Potassium	0.74	0.63	0.91	0.87
Chloride	0.30	0.30	0.30	0.30
DEB[7] (mEq/100g)	198	180	259	254

[1]Trace minerals from premix provided per kilogram of diet: manganese ($MnSO_4$), 120 mg; zinc ($ZnSO_4$), 120 mg; iron ($FeSO_4$), 80 mg; copper ($CuSO_4$), 10 mg; iodine [$Ca(IO_3)_2$], 2.5 mg; and cobalt ($CoSO_4$), 1 mg. Breeder diets contained additional selenium for a total concentration of 0.3 mg/kg of diet.

[2]Vitamins from premix provided per kilogram of diet: vitamin A, 13,228 IU; vitamin D_3, 3,968 IU; vitamin E, 66 IU; vitamin B_{12}, 40 μg; riboflavin, 13 mg; niacin, 110 mg; D-pantothenic acid, 22 mg; menadione, 4 mg; folic acid, 2 mg; vitamin B_6, 8 mg; thiamine, 4 mg; and biotin, 250 μg. Breeder diets contained additional biotin for a total concentration of 375 μg/kg of diet.

[3]Ionophore monensin at 90 g/ton (Coban 90, Elanco Animal Health, Greenfield, IN).

[4]BMD = bacitracin methylene disalicylate at 60 g/ton (BMD 60, Alpharma Inc, Animal Health, Bridgewater, NJ).

[5]Ronozyme P CT at 185 g/ton to provide 925 FYT (DSM Nutritional Products, Parsippany, NJ).

[6]Diets formulated on total amino acid basis for breeders and on ileal digestible amino acid basis for broilers. The digestibility coefficients were obtained from Ajinomoto Heartland: True digestibility of essential amino acids for poultry, Revision 7, Ajinomoto Heartland LLC, Chicago, IL.

[7]DEB = dietary electrolyte balance.

were formulated to have similar nutrient composition for each feed type. Nutrient specifications followed commercial practices and recommendations for the parent stock from 1 of the genetic companies (Cobb-Vantress, 2008). Supplemental biotin was added to all diets to meet recommended levels (Cobb-Vantress, 2008). All pullet diets were offered in mash form and breeder diets were pelleted. Diets used during the laying phase are shown in Table 1. Breeders were vaccinated against NDV and infectious bronchitis virus (**IBV**) with 4 live vaccines administered orally via drinking water. The strain type-1 Hitchner B1 (Triplevac, Intervet, Millsboro, DE) was applied at 2 wk, and La Sota NDV strain vaccines (Combovac, Intervet) were administered at 5, 11, and 18 wk of age. Both vaccines contained Massachusetts and Connecticut types of IBV.

At 22 wk, all birds were weighed, and within each treatment, pullets were classified into 8 sequential BW categories of equal numbers of birds. At 23 wk, 4 pullets were taken at random from each BW category, within each 1 of the 4 treatments (strain × dietary grain source), and moved to a cage breeder house where they were placed at a cage density of either 1 or 2 hens/cage. As a result, 128 pullets were moved to cages to have 16 hens per each of the 8 treatment combinations. This distribution guaranteed that weight differences

between birds in both cage-density treatments (1 or 2 hens/cage) were similar across each strain by dietary treatment combination. Cage size was 1,350 cm^2 (30 × 45 cm) providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space and 2 nipple drinkers per cage. This single or double cage-density factor was a composite stressor that included confounding effects of cage, feeder space availability per bird, and social interactions, as might be encountered under practical conditions, but was used to evaluate the general effects of the presence or absence of competition. Therefore, the factorial effects of genetics (strains A and B), dietary grain source (corn and wheat), and cage density (1 or 2 hens/cage) were evaluated in a factorial arrangement (2 × 2 × 2) of treatments, resulting in 8 breeder treatment combinations.

Breeder Data Collection

Breeder BW were obtained at 24, 40, and 51 wk of age. Possible effects of nutritional or management stressors were evaluated using relative asymmetry (**RA**) at 39 wk according to Møller and Swaddle (1997), and heterophil to lymphocyte ratio (**H:L**) at 53 wk as described by Gross and Siegel (1983). Lengths (0.1 mm) of the left and right shanks were measured at 39 wk of age, with the left-right order being randomized to remove systematic biases (David et al., 1999). Blood, obtained from each hen at 53 wk of age, was used to make smears and also mixed with sodium heparin as an anticoagulant to obtain plasma. Blood smears were prepared for determining the number of heterophils and lymphocytes. All slides were coded and approximately 100 cells on each slide were classified by the same individual. Blood plasma obtained from 12 hens per treatment was analyzed for NDV by ELISA using a ProFlock antibody test Kit (IDEXX NDV antibody test kit, IDEXX Laboratories Inc., Totowa, NJ) with modifications, as described by Hamal et al. (2006).

Data Collection in Chicken Progeny

Two experiments were conducted with the progeny of these breeders, which were artificially inseminated at 44 (experiment 1) and 52 wk of age (experiment 2). Eggs were collected for 8 d, stored for 2 d, set in a Chick Master incubator (model G18, Chick Master Incubator Co., Medina, OH), and placed in individual pedigree bags at d 19 of incubation. In experiment 1, blood samples of 5 chicks per treatment combination were collected at hatching. Serum was isolated and stored at −20°C until analyzed. Serum antibody titers for NDV were determined by ELISA using a ProFlock antibody test Kit (IDEXX NDV antibody test kit), according to the manufacturer's directions. All samples were analyzed in duplicate, with each plate containing negative and positive controls. The optical density (**OD**) of plates was measured at a wavelength of 650 nm using a microplate reader (Molecular Devices Inc., Sunnyvale, CA). The mean OD data of each sample was used for statistical analyses to avoid mathematical manipulations that may add correlation to the data and could increase the error to estimate true effects of breeder treatments; however, ELISA units were calculated for data discussion, according to Hamal et al. (2006).

In experiment 2, eggs were collected for 6 d and stored at 15°C and 65% RH. Eggs were placed in individual pedigree bags at d 19 of incubation. At hatching, chicks were individually neck tagged and 12 chicks for each of the 8 treatment combinations were placed in battery cages in an isolation room. The chicks were not vaccinated, and no medication was given during the 13-d study period. Chicks were given 24 h of light through the first week of age and 20 h thereafter. Progeny were fed ad libitum the same starter dietary grain type, corn- or wheat-based, as their parents had been fed because this is the common practice in those regions of the world where each grain source is more available for poultry feed. Broiler diets (Table 1) were also formulated to have similar nutrient composition for each grain type. Blood samples from 5 chicks per treatment combination were collected at hatching, 5, 9, and 13 d of age. The serum antibody levels against NDV were assessed by ELISA. Bursa and spleen were collected at hatching from the same chicks as were bled and weighed to the nearest 0.001 g. These tissues were fixed in Bouin's fluid. Transversal histological cuts from the median region of each tissue were stained with hematoxylin and eosin. The histological structures of the lymphoid tissues were observed using a light microscope (5× magnification). Measurements of different histological structures of the lymphoid tissues were performed using a calibrated stage micrometer in micrometers (UTHSCSA ImageTool software Version 3.0; University of Texas Health Science Center, Department of Dental Diagnostic Science, San Antonio, TX).

Statistical Analyses

The experimental units were individual hens or progeny chicks randomly sampled from each treatment group. The RA of lengths and diameters of the left (**L**) and right (**R**) shanks was defined as the ratio of the absolute value of asymmetry (L – R) divided by the size of the bilateral trait: $RA = \{(L - R)/[(L + R)/2]\} \times 100$ (Møller and Swaddle, 1997). The OD data of antibody titers were log transformed and the percentage of spleen white-pulp data were transformed to arcsin prior to statistical analysis. Data were analyzed as a 2 × 2 × 2 factorial design considering strain, dietary grain source, and cage density as main factors. Data were subjected to ANOVA using JMP 8.0 (SAS Institute Inc., 2009). Additionally, the effect of chick age was included in the statistical model to evaluate the overall effect of treatments on MatAb catabolism. Differences between treatment means were evaluated using Tukey's test. Statements of statistical significance were based upon $P \leq 0.05$. Results are presented as means ± SEM.

Table 2. Probabilities of sources of variation on breeder BW, relative asymmetry (RA) of shank length (SL) at 39 wk, heterophil to lymphocyte ratio (H:L), and optical density (OD) for anti-Newcastle disease virus (NDV) antibodies at 53 wk

	BW[1]				Stress parameter		
Source of variation	22 wk	24 wk	40 wk	51 wk	RA SL	H:L	ELISA NDV (OD)
Strain[2]	0.534	0.174	0.725	0.704	0.749	0.471	0.485
Dietary grain source[3]	0.003	0.036	0.014	0.952	0.037	0.525	0.146
Cage density[4]	0.139	0.146	0.101	0.085	0.046	0.863	0.079
Strain × breeder diet	0.508	0.886	0.442	0.295	0.728	0.385	0.193
Strain × cage density	0.122	0.409	0.034	0.035	0.823	0.044	0.986
Diet × cage density	0.467	0.378	0.516	0.662	0.062	0.396	0.007
Strain × diet × cage	0.351	0.356	0.024	0.053	0.835	0.113	0.292

[1]Body weight of breeder hens (n = 15).

[2]Broiler breeder females of commercial strains A and B were housed in floor pens during the rearing phase and cages during the egg-laying phase.

[3]Breeders were fed either corn- or wheat-based diets formulated to be isonutrient during rearing and egg-laying phases.

[4]At 23 wk, pullets and cocks that represented the BW distribution from each treatment were moved to a cage breeder house and placed at either 1 or 2 hens/cage. Cage size was 1,350 cm^2 (30 × 45 cm) providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space. This single or double cage density was used to evaluate the general effects of competition.

RESULTS AND DISCUSSION

Breeder BW, RA, H:L Ratio, and Antibodies

Hen BW were only affected by dietary grain source at 22 ($P < 0.01$) and 24 ($P < 0.05$) wk (Table 2). Dietary grain source also affected breeder BW at 40 wk ($P < 0.05$), but not at 51 wk of age. Breeders fed corn-based diets were smaller than hens fed wheat-based diets at 22, 24, and 40 wk (Table 3). These diets were formulated to have a similar nutrient composition (Table 1). However, results indicated that during rearing, corn-based diets were not able to support growth rates similar to those observed in hens fed wheat-based diets. The main nutrients (protein, fat, calcium, and phosphorus) were confirmed by laboratory analyses to be similar to formulated values, but the ME was not evaluated. Additional fat was used in wheat-based diets to obtain isoenergetic diets, and consequently, the effective ME value and the fatty acid profile may have changed. At 40 and 51 wk, a 3-way interaction effect (strain × dietary grain source × cage density) was observed ($P < 0.05$) for hen BW (Table 2). These effects were similar to the ones observed in the strain by cage-density interaction effect ($P < 0.05$), and these were selected to simplify data discussion. At 40 wk, breeders of strain B housed at 2 hens/cage were heavier than those housed at 1 hen/cage, without being significantly different from hens of strain A (Table 4). At 51 wk, strain A hens housed at 2 hens/cage were smaller than those housed at 1 hen/cage, and not different from strain B hens (Table 4). Even though these interactive effects were variable, they indicated the importance of competition on hen nutrient intake, growth, and development in both strains.

These effects of cage density were also confirmed by the RA at 39 wk and the H:L at 53 wk. The RA of shank length at 39 wk was affected ($P < 0.05$) by dietary grain source and cage density (Table 2 and 3). Higher RA were observed in breeders fed corn-based

Table 3. Effect of main factors on breeder BW, relative asymmetry (RA) of shank length (SL) at 39 wk, and optical density (OD) for anti-Newcastle disease virus (NDV) antibodies at 53 wk

	BW (g)[1]					
Main factor	22 wk	24 wk	40 wk	51 wk	RA SL	ELISA NDV (OD)[2]
Strain[3]						
A	1,993 ± 37	2,440 ± 46	3,778 ± 62	4,000 ± 67	2.46 ± 0.23	0.195 ± 0.027
B	2,027 ± 39	2,532 ± 49	3,770 ± 67	3,962 ± 72	2.26 ± 0.26	0.187 ± 0.027
Dietary grain source[4]						
Corn	1,928 ± 39[b]	2,415 ± 49[b]	3,640 ± 66[b]	3,984 ± 72	2.72 ± 0.24[a]	0.173 ± 0.023
Wheat	2,092 ± 37[a]	2,557 ± 47[a]	3,869 ± 63[a]	3,978 ± 68	2.01 ± 0.23[b]	0.209 ± 0.030
Cage density[5]						
1 hen/cage	1,969 ± 41	2,437 ± 50	3,679 ± 68	4,067 ± 74	2.10 ± 0.25[b]	0.195 ± 0.027
2 hens/cage	2,040 ± 36	2,536 ± 45	3,830 ± 61	3,895 ± 66	2.62 ± 0.23[a]	0.187 ± 0.027

[a,b]Means within a column followed by different superscripts are significantly different ($P < 0.05$).

[1]Body weight of breeder hens (n = 16).

[2]The anti-NDV antibody levels were determined using an NDV ELISA test kit in breeder plasma samples collected at 53 wk. The OD of the negative and positive controls were 0.051 and 0.283.

[3]Broiler breeder females of commercial strains A and B were housed during the rearing phase in floor pens and cages during the egg-laying phase.

[4]Breeders were fed either corn- or wheat-based diets formulated to be isonutrient during rearing and egg-laying phases.

[5]At 23 wk, pullets and cocks that represented the BW distribution from each treatment were moved to a cage breeder house and placed at either 1 or 2 hens/cage. Cage size was 1,350 cm^2 (30 × 45 cm) providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space. This single or double cage density was used to evaluate the general effects of competition.

Table 4. Interactive effect of strain and cage density on breeder BW at 40 and 51 wk, heterophil:lymphocyte (H:L) ratio, and optical density (OD) for anti-Newcastle disease virus (NDV) antibodies at 53 wk

	Strain A		Strain B	
Source of variation	1 hen/cage	2 hens/cage	1 hen/cage	2 hens/cage
BW				
40 wk	3,761 ± 91[ab]	3,716 ± 85[ab]	3,597 ± 102[b]	3,944 ± 87[a]
51 wk	4,192 ± 99[a]	3,807 ± 93[b]	3,942 ± 110[ab]	3,982 ± 94[ab]
H:L ratio, 53 wk	1.16 ± 0.06[ab]	1.29 ± 0.54[a]	1.24 ± 0.07[ab]	1.13 ± 0.05[b]
ELISA NDV (OD), 53 wk[1]	0.235 ± 0.044	0.155 ± 0.031	0.207 ± 0.036	0.168 ± 0.041

[a,b]Means within a row followed by different superscripts are significantly different ($P < 0.05$; n = 16).

[1]The anti-NDV antibody levels were determined using an NDV ELISA test kit in breeder plasma samples collected at 53 wk. The OD for the negative and positive controls were 0.051 and 0.283, respectively.

diets than in those fed wheat-based diets, and in hens housed at 2 hens/cage than in hens housed at 1 hen/cage. The strain by cage-density interaction had an effect ($P < 0.05$) on H:L (Table 2). Strain A hens housed at 2 hens/cage had higher H:L than strain B hens housed at 2 hens/cage, but these values were not different from those observed in hens of both strains housed at 1 hen/cage (Table 4). Strain A had the highest levels of stress when housed at 2 hens/cage. The H:L observed in this experiment were similar to those reported by Gross and Siegel (1983) for high social-stress birds. The cage-density factor was a composite stressor without severe restrictions on space, feeder space, or water space. Under commercial conditions, the feeder space available to the breeder could be between 15 and 9 cm/hen, which is less than what we provided in the present study. This treatment helped to evaluate 2 well-defined levels of social interactions (1 vs. 2 hens/cage). Under floor pens or commercial conditions with several dozens or thousands of birds, there are multiple levels of social interactions that may cause similar or higher levels of stress (Rosales, 1994), but it would be more difficult to detect and analyze.

The anti-NDV antibodies (OD) of hens at 53 wk were affected by the interactive effect ($P < 0.01$) of dietary grain source by cage density (house 2). Hens fed corn-based diets had higher levels of antibodies when housed at 1 hen/cage than at 2 hens/cage, but no significant effect of cage density was observed when hens were fed wheat-based diets (Figure 1).

Maternal Antibody Transfer

In both experiments (Table 5), as shown by the strain by cage-density interaction, chicks at hatching from breeders of strain A placed at 2 hens/cage had lower ($P < 0.05$) NDV titers at hatching than chicks from hens of the same strain placed at 1 hen/cage (Figure 2). In contrast, the MatAb levels of strain B chicks at hatching did not significantly differ when breeders were placed at 1 or 2 hens/cage. Progeny of strain B housed at 2 hens/cage had MatAb titers similar to chicks from strain A breeders placed at 1 hen/cage in both experiments. In experiment 1, progeny of strain B breeders placed at 1 hen/cage had higher MatAb levels than the chicks of strain-A breeders housed at 2 hens/cage, but in experiment 2, chicks of those treatments had similar MatAb levels.

Breeder dietary grain source had no significant effect on MatAb transfer to progeny (Table 5). The level of MatAb deposited in the yolk, and subsequently found in circulation in the chick, has previously been dem-

Table 5. Probabilities of sources of variation on optical density (OD) for anti-Newcastle disease virus (NDV) maternal antibodies at hatching on experiment 1 and experiment 2, and on areas of bursa follicles and white pulp of spleen at hatching in experiment 2[1]

	Experiment 1	Experiment 2		
Source of variation	ELISA NDV (OD)	ELISA NDV (OD)	Area of follicles (bursa)	Area of white pulp (spleen)
Strain[2]	0.039	0.4168	0.412	0.011
Dietary grain source[3]	0.356	0.9118	0.159	0.002
Cage density[4]	0.089	0.1975	0.001	0.053
Strain × breeder diet	0.115	0.7089	0.127	0.010
Strain × cage density	0.017	0.0126	0.001	0.254
Diet × cage density	0.609	0.1989	0.258	0.033
Strain × diet × cage density	0.543	0.5952	0.001	0.625

[1]The chicks were hatched from consecutively laid fertile eggs collected when the hens were either 44 (experiment 1) or 52 (experiment 2) wk of age (n = 5).

[2]Broiler breeder females of commercial strains A and B were housed during the rearing phase in floor pens and cages during the egg-laying phase.

[3]Breeders were fed either corn- or wheat-based diets formulated to be isonutrient during rearing and egg-laying phases.

[4]At 23 wk, pullets and cocks that represented the BW distribution from each treatment were moved to a cage breeder house and placed at either 1 or 2 hens/cage. Cage size was 1,350 cm^2 (30 × 45 cm) providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space. This single or double cage density was used to evaluate the general effects of competition.

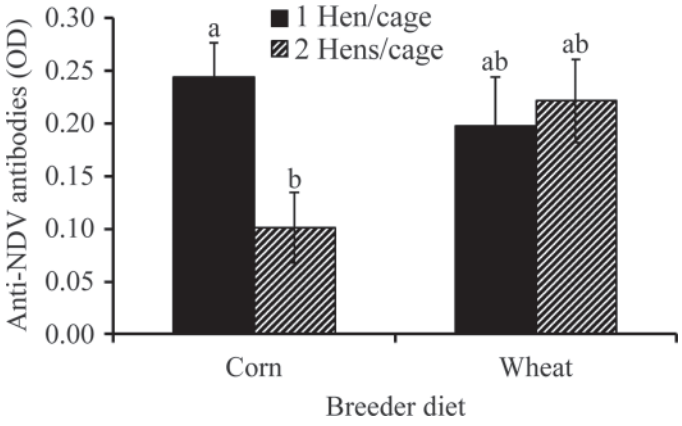

Figure 1. Interaction effect of breeder dietary grain source and cage density on breeder hen anti-Newcastle disease virus (NDV) antibody levels (optical density; OD) in plasma measured at 53 wk. Breeders were administered 4 live vaccines against NDV during rearing in litter floor pens and housed in cages during the egg-laying phase. At 23 wk, pullets and cocks were moved to a cage breeder house and placed at either 1 or 2 hens/cage. Cage size was 1,350 cm^2 (30 × 45 cm), providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space. This single or double cage density was used to evaluate the general effects of competition. The anti-NDV antibody levels were determined using an NDV ELISA test kit. Data represent OD means of 14 samples, with 7 samples per treatment combination. Error bars correspond to SEM. Letters denote significant ($P < 0.05$) differences among treatments. The OD of the negative and positive controls for experiment 1 were 0.051 and 0.283.

onstrated to be proportional to the level of circulating antibodies in the hen (Rahman et al., 2002; Hamal et al., 2006). This suggested the differences in anti-NDV antibodies found in the chicks at hatching were the result of cage-density effects on the hen's ability to produce antibodies. The second experiment was conducted with eggs collected at 52 wk, and at this age, dietary grain source did not affect breeder BW or hen anti-NDV antibodies. However, when fed corn-based diets, breeders housed at 2 hens/cage had lower anti-NDV antibody levels than hens housed at 1/hen cage. Cage density at 2 hens/cage was an environmental composite stressor in strain A breeders, as evidenced by higher H:L. Consequently, lower antibody titers of chicks coming from hens of strain A housed at 2 hens/cage could be because of either a higher degree of stress of the breeder or lower availability of nutrients caused by the competition that affects hens of strain A more than hens of strain B (Table 4). Siegel (1985) reported that stress caused decreased antibody responses to vaccination. However, in the present experiment, it was difficult to determine whether competition (cage density), altered nutrient intake, or both were the cause of lower MatAb transfer. It was interesting to observe that these 2 strains responded differently to cage-density conditions, and regardless of breeder age (44 or 52 wk in experiments 1 and 2, respectively), cage density influenced the transfer of antibodies to the progeny. Hamal et al. (2006) had reported differences in MatAb transfer between 2 genetic lines; however, in that experiment, breeder hens were housed in different farms. The present data indicated that breeder cage-density conditions may play an important role in MatAb transfer to offspring.

Maternal Antibody Catabolism

In experiment 2, MatAb against NDV declined in chick serum as these aged ($P < 0.001$). The catabolism, or disappearance, of MatAb were affected ($P < 0.05$) by the interaction between hen strain and cage density. This interactive effect was observed in the overall model, using chick age as the covariate, and in the evaluation of each sampling time (Table 6). Additionally, in the evaluation of each sampling time, a dietary grain source by cage-density interactive effect was observed

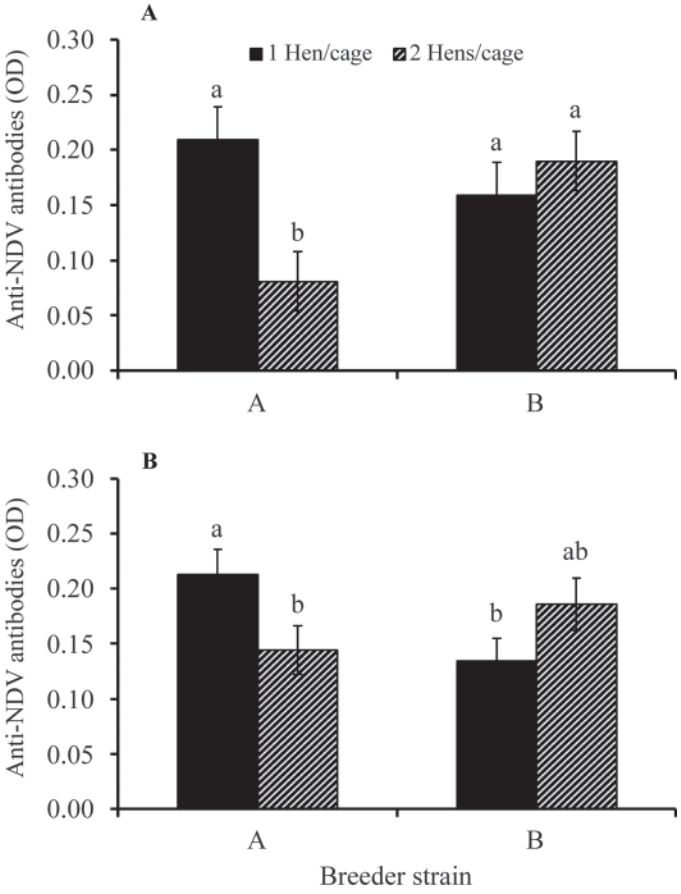

Figure 2. Interaction effect of breeder strain and cage density on maternal anti-Newcastle disease virus (NDV) antibody levels (optical density; OD) in chick serum at hatching in experiment 1 (panel A) and experiment 2 (panel B). The chicks were hatched from consecutively laid fertile eggs collected when the hens were either 44 (experiment 1) or 52 (experiment 2) wk old. Breeders were administered 4 live vaccines against NDV during rearing in litter floor pens and housed in cages during the egg-laying phase. At 23 wk, pullets and cocks were moved to a cage breeder house and placed at either 1 or 2 hens/cage. Cage size was 1,350 cm^2 (30 × 45 cm), providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space. This single or double cage density was used to evaluate the general effects of competition. The anti-NDV antibody levels were determined using an NDV ELISA test kit. Data represent OD means of 10 samples, 5 samples per treatment combination. Error bars correspond to SEM. Letters denote significant ($P < 0.05$) differences among treatments. The OD of the negative and positive controls for experiment 1 were 0.058 and 0.479. The OD for the negative and positive controls for experiment 2 were 0.048 and 0.513.

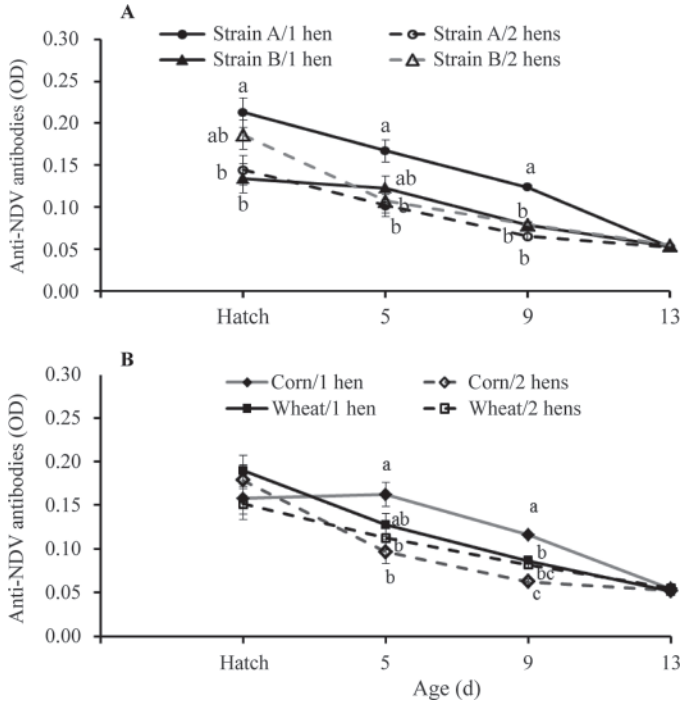

Figure 3. Interaction effects of breeder strain and cage density (panel A) and breeder dietary grain source and cage density (panel B) on maternal anti-Newcastle disease virus (NDV) antibody levels (optical density; OD) in progeny chick serum from hatching to 13 d of age (experiment 2). The chicks were hatched from consecutively laid fertile eggs collected when the hens were 52 wk old. Breeders were administered 4 live vaccines against NDV during rearing in litter floor pens and housed in cages during the egg-laying phase. The chicks were raised in battery cages in an isolation room, not vaccinated, and no medication was given during the 13-d study period. The anti-NDV antibody levels were determined using an NDV ELISA test kit. Each data point represents OD means of 10 samples, 5 samples per treatment combination for each sampling time. Error bars correspond to SEM. Letters denote significant ($P < 0.05$) differences among treatments within each time point.

at 5 and 9 d of age (Table 6) in the chicks, but no significant effects of breeder treatments were observed at 13 d of age. It was expected (Rahman et al., 2002; Hamal et al., 2006) that anti-NDV MatAb would practically not be detectable (mean OD = 0.051) at 13 d of age (negative control OD = 0.041). Broiler progeny from strain A breeders had lower NDV antibody titers when hens were housed at 2 hens/cage compared with at 1 hen/cage (Figure 3A) at 5 and 9 d. In contrast, progeny from strain B breeders exhibited similar MatAb levels independent of cage density. The levels of NDV MatAb of chicks from strain B breeders housed at 2 hens/cage were similar to the levels observed in progeny from strain A breeders housed at 2 hens/cage at hatching and 5 d of age. These results suggested that the rate of NDV MatAb degradation was similar in chicks from these treatment combinations, given that the observed titers follow the same order that was observed at hatching.

Chicks from breeders fed corn-based diets and housed at 2 hens/cage had lower ($P < 0.05$) titers when compared with progeny of breeders fed similar diets but housed at 1 hen/cage (Figure 3B). However, no significant effect of cage density was observed in the chick groups fed wheat-based diets. This effect of dietary grain source was observed only at 5 and 9 d of age, indicating a possible effect of chick diet on rate of NDV MatAb degradation. Kidd et al. (1993) showed that grain source, corn-soybean or milo-corn-soybean, in diets of broiler breeders resulted in different levels of primary antibody response to sheep red blood cells in progeny. Nevertheless, the same interactive effect of dietary grain source by cage density was observed for anti-NDV antibody levels in breeder hens as for their progeny (Figures 1 and 3).

Lymphoid Tissue Development

No significant effects of treatments were detected in bursa or spleen tissue weights at hatching. The results of histological evaluations indicated that there were interaction effects ($P < 0.05$) of breeder strain and dietary grain source (Figure 4A), and dietary grain source and cage density (Figure 4B) on percentage of spleen white pulp from chicks at hatching. Chicks from strain A breeders fed corn-based diets exhibited a lower

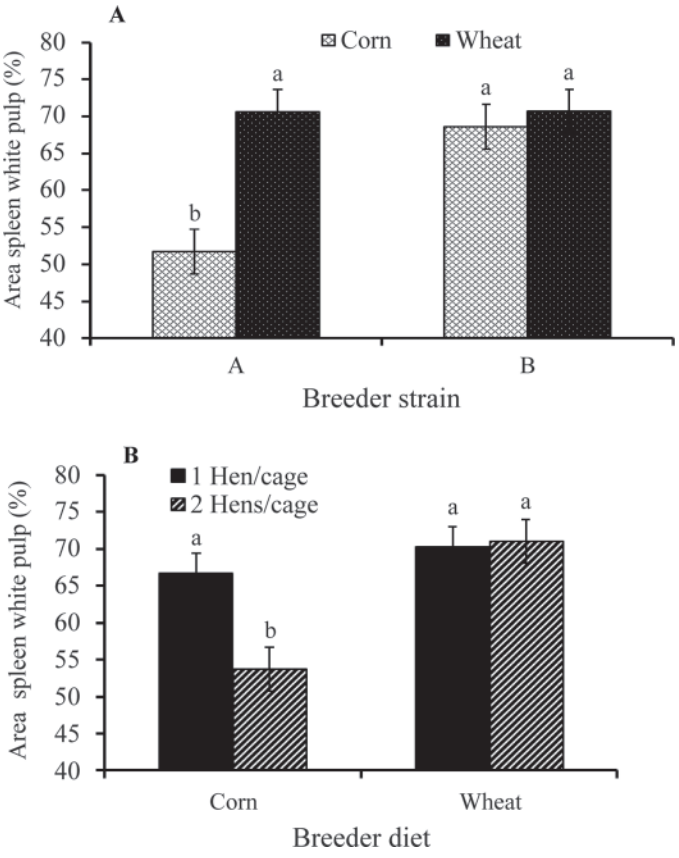

Figure 4. Interaction effects of breeder strain and dietary grain source (panel A) and dietary grain source and cage density (panel B) on spleen white pulp (%) from chicks at hatch (experiment 2). The chicks were hatched from consecutively laid fertile eggs collected when the hens were 52 wk old. Breeders were raised in litter floor pens and housed in cages during the egg-laying phase and fed corn- or wheat-based diets. Data represent means of 10 samples, 5 samples per treatment combination. Error bars correspond to SEM. Letters indicate significant ($P < 0.05$) differences among treatments.

Table 6. Probabilities of sources of variation on optical density (OD) for anti-Newcastle disease virus (NDV) maternal antibodies measured in chick progeny at 5, 9, and 13 d of age in experiment 2[1]

Source of variation for ELISA NDV OD	Chicken age (d)		
	5	9	13
Strain[2]	0.099	0.019	0.223
Dietary grain source[3]	0.411	0.438	0.706
Cage density[4]	0.002	<0.001	0.413
Strain × breeder diet	0.006	0.187	0.211
Strain × cage density	0.039	<0.001	0.772
Diet × cage density	0.038	0.001	0.431
Strain × diet × cage density	0.004	0.843	0.729

[1]The chicks were hatched from consecutively laid fertile eggs collected when the hens were 52 wk of age. Five serum samples per treatment combination were analyzed at each time point.

[2]Broiler breeder females of commercial strains A and B were housed in floor pens during the rearing phase and cages during the egg-laying phase.

[3]Breeders were fed either corn- or wheat-based diets formulated to be isonutrient during rearing and egg-laying phases.

[4]At 23 wk, pullets and cocks that represented the BW distribution from each treatment were moved to a cage breeder house and placed at either 1 or 2 hens/cage. Cage size was 1,350 cm^2 (30 × 45 cm) providing either 30 cm (1 hen/cage) or 15 cm (2 hens/cage) of feeder space. This single or double cage density was used to evaluate the general effects of competition.

percentage of spleen white pulp than that from other treatments ($P < 0.05$). Breeders fed corn-based diets and housed at 2 hens/cage produced chicks with a lower percentage of spleen white pulp ($P < 0.05$). The area of bursa follicles (mm^2) at hatching was influenced ($P < 0.001$) by interaction effects among breeder strain, dietary grain source, and cage density (Table 5). Chicks from strain A breeders fed wheat and progeny of strain B breeders fed corn had smaller areas of bursa follicles when placed at 2 hens/cage compared with that from progeny of counterpart breeders placed at 1 hen/cage (Figure 5). The smallest bursa follicle areas were observed in chicks from strain A breeders fed wheat-based diets and placed at 2 hens/cage.

These results suggested differences among strains with regard to bursa and spleen development of progeny chicks. This was not surprising as Apanius (1998) discussed the differences in bursa growth among strains of chickens and the correlations observed in the growth of spleen and thymus, antibody responsiveness, and several other measurements of general disease resistance. The data collected in the present experiment indicated that maternal nutrition might affect lymphoid tissue development in the progeny. Several reports (Kidd et al., 1993; Grindstaff et al., 2003; Kidd, 2003; Koutsos et al., 2003; Virden et al., 2004) have indicated that breeder diets may also affect the development of cellular immunity.

In general, progeny of hens from strain A housed at 2 hens/cage had smaller areas of bursa follicles and a lower percentage of spleen white pulp at hatching than those from progeny of breeders housed at 1 hen/cage. Results of breeder hen BW, RA, and H:L indicated that dietary grain source and competition (cage density) may have contributed to this response. In his review, Rosales (1994) reported that corticosteroids in poultry could cause atrophy of the spleen, thymus, and bursa of Fabricius, as well as decreased antibody responses and a decreased numbers of lymphocytes. To our knowledge, this was the first time that these maternal management and dietary effects on immune tissue development of progeny have been examined in the present manner.

In summary, these data demonstrated that cage-density conditions of broiler breeders may have significant effects on the hen's ability to deposit MatAb critical for protecting chicks from infection in the first few weeks of life and can affect how their primary and secondary immune tissues develop in ovo. It has long been recognized in humans and other species that seemingly minor differences in the stress, nutritional, and environmental conditions of the mother can profoundly affect embryo development (Merlot et al., 2008). Additionally, given the different selection programs to which each commercial broiler breeder line has been subjected, it should

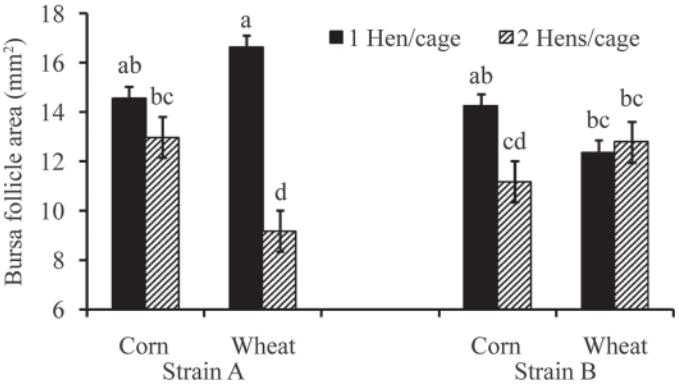

Figure 5. Interaction effects of breeder strain, dietary grain source, and cage density on area of bursa follicles (mm^2) at hatch (experiment 2). The chicks were hatched from consecutively laid fertile eggs collected when the hens were 52 wk old. Breeders were raised in litter floor pens and housed in cages during the egg-laying phase and fed corn- or wheat-based diets. Data represent means of 5 samples per treatment combination. Error bars correspond to SEM. Letters indicate significant ($P < 0.001$) differences among treatments.

not be surprising that different genetic lines were affected differently by changes in dietary grain source and cage density. These effects have been observed in broilers (Cheema et al., 2003), and the data presented herein indicated an effect on broiler breeders as well. Ultimately, these data demonstrated a need for a greater understanding of how seemingly minor changes in breeder management practices affect the overall development and immune competence of specific genetic lines of broiler chickens.

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