

Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures

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Primary Audience: Nutritionists, Researchers, Broiler Supervisors, Broiler Growers

SUMMARY

Feedstuffs and additives with immunomodulatory characteristics were investigated to reduce losses caused by disease and to optimize the production potential of the bird. Therefore, the objective of this study was to assess the influence of yeast extract and prebiotics on broilers fed a prestarter diet, and the impact of ambient temperature on maternal and postvaccinal humoral immune responses. The birds were fed diets supplemented with yeast extract, prebiotics, or both in the prestarter phase (1 to 7 d), and from d 8 on, all birds received the same diet. The birds were vaccinated against Newcastle disease virus and infectious bursal disease at 8 and 18 d of age. The addition of yeast extract and prebiotic in the prestarter phase did not change the humoral immune response against Newcastle disease virus or infectious bursal disease throughout the production cycle of the broilers. The higher temperature increased IgG levels, especially maternal antibody titers, despite the immunomodulating effect of heat stress until the second week of life.

Key words: heat stress, infectious bursal disease, Newcastle disease, mannan oligosaccharide, *Saccharomyces cerevisiae*

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DESCRIPTION OF PROBLEM

The immune system of the newly hatched chick is prepared to fight pathogens in a non-specific manner through innate immunity, and can develop specific modes of defense through cellular and humoral immunity, which requires

contact with antigens [1]. Acquired immunity differs from the innate responses by possessing specificity in recognition of foreign invaders (antigens) and the development of memory; therefore, it results in a more rapid response than was elicited during the primary exposure when the bird is reexposed to an antigen [2].

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Broilers have lymphoid structures spread throughout the intestinal tract, and these are made up of diffuse and aggregated components. Diffuse components include intraepithelial lymphocytes as well as lymphocytes of the mucosa and lamina propria; aggregated components include Peyer's patches, cecal tonsils, and the bursa of Fabricius. These tissues capture antigens available in the digestive tract, which stimulate B lymphocyte-derived plasma cell precursors of IgM, IgG, and IgA, and T and B lymphocytes, which activate Peyer's patches [3, 4] to enhance general and specific immunity.

The immunological stimulation of the mucosa favors the production of IgA antibodies [5–7], mainly in Peyer's patches [8], which block receptors [9–11] and reduce the number of pathogenic bacteria in the intestinal lumen [12, 13]. The constant production of IgA secreted in large amounts on the surface of the intestinal mucosa occurs because of the continuous stimulation of normal gut microflora [14, 15].

Research has shown that the Harderian gland has large amounts of IgA and B lymphocytes on the surface, which perform a significant migration process to cecal tonsils and, at a lower rate, to the bursa of Fabricius. These data suggest a potential connection between the gut-associated immune system and the ocular immune system [16, 17].

Dietary bioactive food components that interact with the immune response have considerable potential to reduce susceptibility to infectious diseases. In fact, because of the genetically homogenous populations of domestic fowl in the commercial environment, using nutritional dietary intervention strategies could prove to be a cost-effective means of preventing specific infectious diseases and maintaining the health of poultry flocks [18].

Yeast extract is a protein source derived from the cell content of live yeast. It has high nucleotide, inositol, and glutamic acid levels [19]. In previous studies, the addition of yeast extract of *Saccharomyces cerevisiae* to broiler prestarter diets resulted in beneficial effects on the feed conversion of the birds at 21 d of age [20, 21]. Nucleotides are traditionally used in human diets, particularly in infant diets; they favor the development of the gastrointestinal tract and the immune functions, and they maintain the gut

flora [22]. Further studies are required to explain the mechanism through which the immune system is influenced by dietary nucleotides. Although the majority of nucleotides in the diet are metabolized rapidly and then excreted, a significant amount of those retained are found in the gastrointestinal tissue [23]. Several factors, such as food restriction, rapid growth, and certain diseases, may interfere in the endogenous synthesis of these nucleotides; thus, the nucleotide becomes essential under these conditions [24]. Jyonouchi [23] investigated the impact of nucleotides on the immune response in vitro and determined its importance in maintaining the optimal conditions required for the humoral immune response. According to the author, nucleotide deficiency damages cell immunity, and supplementation is therefore recommended.

The positive effect of adding prebiotics to broiler diets on bird performance has been already demonstrated. In a survey conducted to investigate the effects of including mannan oligosaccharides in the prestarter diet of broilers reared under different temperatures (18, 25, and 32°C) [20], improvements in BW gain (5.11%) were observed in birds reared under a high temperature to 42 d of age and improvements in livability (2.80%) were observed until 21 d of age.

By promoting the growth of lactic acid-producing bacteria, prebiotics have an indirect, beneficial effect on the immune system of the host. These bacterial populations produce immunostimulating substances that react with the immune system at different levels, including the production of cytokines, mononuclear cells, and macrophage phagocytosis as well as the induction of synthesis of large amounts of Ig, particularly IgA [25, 26].

Newcastle disease is a contagious, lethal infection that affects all birds. It is caused by *Paramyxovirus* type 1 (Newcastle disease virus; **NDV**) [27, 28]. The maternal antibodies protect the chick during the first week of life and may interfere in the development of humoral immunity. However, they cannot invalidate the rapid establishment of vaccine protection [29].

Infectious bursal disease (**IBD**), also known as Gumboro, is an infectious, contagious disease that affects the bursa of Fabricius, a key organ in the development of the immune system of young birds [30]. In 1-d-old chicks, maternal antibody

Table 1. Temperatures of the chambers (mean ± SD)

Age, d	Chamber temperature, °C		
	High	Control	Low
1 to 3	35 ± 1	32 ± 2	28 ± 3
4 to 7	34 ± 1	31 ± 1	26 ± 2
8 to 14	32 ± 3	28 ± 2	22 ± 2
15 to 21	31 ± 3	26 ± 2	20 ± 3
22 to 42	30 ± 3	23 ± 2	19 ± 2

levels against IBD are high and remain so until d 7, and then they are reduced by approximately 35 d of age. Although these antibodies may be detected until wk 4 of life, protection limits are exhausted in the second week of life [31, 32].

It is widely known that ambient temperatures not only can change broiler susceptibility to infectious diseases, but also can affect the humoral immune response [33]. The effect of ambient temperature on immune response can vary according to the ability of the bird to maintain homeothermy [34]. Exposure of birds to adverse environmental conditions generates adaptive responses such as acclimation, which involves a series of reactions that peak with glucocorticoid secretion [35]. However, it is well known that the action of glucocorticoid is antagonistic to the development of the immune response in birds [36], thereby impairing the expression of the humoral immune response [37].

Considering this, the objective of this study was to assess the effect of yeast extract and prebiotic in a prestarter diet for broilers vaccinated against IBD and NDV, and raised at different temperatures, on maternal and postvaccinal humoral immune response.

MATERIALS AND METHODS

Birds, Diets, and Management

The experiment was conducted in the experimental poultry house of the Department of Animal Science of the Universidade Estadual Paulista, Jaboticabal Campus, with 1,440 one-day-old male Cobb 500 [38] chicks. The birds were raised in 3 climatic chambers, 1 for each brooding temperature, and chambers were lined with polyurethane and had a heating-cooling system. The chambers were divided into 16 boxes with 2.5 × 1.0 m each. Birds were submitted to

different temperatures, as shown in Table 1, and received water and feed ad libitum throughout the experimental period of 42 d.

The experiment was fully randomized in a factorial design with subdivided plots (3 × 2 × 2 factorial design in the plots), using as factors brooding temperatures (high, control, and low), yeast extract levels (0 and 2%), and prebiotic levels (0 and 0.15%), and with subplots, using ages at blood and tear secretion collection (7, 14, 21, 28, 35, and 42 d of age). Four replicates were used with 30 birds/treatment replicate, totaling 12 treatments.

All the diets (in mash) were either unsupplemented or supplemented with yeast extract and prebiotic and were offered only in the prestarter phase (1 to 7 d). Beginning on d 8, all birds in each treatment were fed the same feed, adjusted according to the breeding phase (1 to 7 d, 8 to 21 d, and 22 to 42 d), as recommended by Rostagno et al. [39] (Table 2). The levels of yeast extract and prebiotic followed the directions of the product supplier. The diets were as follows: 1) diet 1: 0% yeast extract and 0% prebiotic; 2) diet 2: 2.0% yeast extract and 0% prebiotic; 3) diet 3: 0% yeast extract and 0.15% prebiotic; 4) diet 4: 2.0% yeast extract and 0.15% prebiotic.

The supplements used in the diets were obtained from the processing of the yeast *S. cerevisiae* strain 1026. The mannan oligosaccharides [40] are derived from the yeast cell wall, and the yeast extract [41] is obtained after removal of the cell wall of yeast by processing with proteolytic enzymes.

At 8 d of age, the birds were vaccinated with live vaccine virus against NDV (La Sota strain) and IBD (intermediate strain Lukert), and at 18 d, the birds were vaccinated against IBD (strong strain Australia V-877). All vaccines were administered ocularly, diluted according to the recommendations of the manufacturer, at 30 mL/1,000 vaccine doses, or 30 µL/bird, based on the method described by Paulillo [42].

Analytical Procedures

Blood samples for antibody analysis were taken by puncture of the brachial vein, and for IgA analysis, the lacrimal secretions were collected by irritating the eye of the bird with glycerin. The samples were always taken from the same broilers by plot, at 7, 14, 21, 28, 35, and

Table 2. Perceptual composition, chemistry composition, and energy value of the experimental diets in the phases at 1 to 7 d, 8 to 21 d, and 22 to 42 d of age

	1 to 7 d					
Item	1 ¹	2	3	4	8 to 21 d	22 to 42 d
Ingredient						
Corn	52.65	52.85	52.77	52.85	54.98	58.38
Soybean meal	39.19	37.03	39.16	37.03	36.63	31.14
Dicalcium phosphate	1.94	1.96	1.93	1.95	1.84	1.86
Limestone	0.93	0.87	0.87	0.87	0.87	0.83
Vegetable oil	3.37	3.25	3.32	3.25	4.15	5.57
Salt	0.52	0.52	0.52	0.52	0.50	0.55
Mineral and vitamin mix ^{2,3,4}	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine (99%)	0.39	0.42	0.38	0.41	0.27	0.21
L-Lysine (78%)	0.37	0.45	0.37	0.45	0.21	0.23
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05
Inert	—	0.10	—	—	—	—
Yeast extract ⁵	—	2.00	—	2.00	—	—
Prebiotic ⁶	—	—	0.15	0.15	—	—
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
ME, kcal/kg	2,960	2,960	2,960	2,960	3,050	3,200
CP, %	21.11	21.14	21.11	21.11	21.14	19.20
Digestible lysine, %	1.33	1.33	1.33	1.33	1.15	1.00
Digestible methionine, %	0.52	0.52	0.52	0.52	0.45	0.41
Digestible methionine + cysteine, %	0.94	0.94	0.94	0.94	0.82	0.72
Calcium, %	0.94	0.92	0.92	0.92	0.90	0.81
Available phosphorus, %	0.47	0.47	0.47	0.47	0.45	0.40
Sodium, %	0.21	0.21	0.21	0.21	0.19	0.19

¹Diets: 1 = control diet; 2 = 2.0% yeast extract; 3 = 0.15% prebiotic; 4 = 2.0% yeast extract and 0.15% prebiotic.

²Mineral and vitamin supplement levels per kilogram: Se, 54.6 mg; Cu, 25,000 mg; calcium pantothenic, 1,900 mg; Mn, 15,252 mg; I, 260 mg; Zn, 18,250 mg; nicotinic acid, 6,930 mg; biotin, 32 mg; DL-methionine, 340 g; choline, 120 g; vitamin A, 1,400,000 IU; vitamin B₁, 356 mg; vitamin B₁₂, 2,000 µg; vitamin B₂, 1,920 mg; vitamin B₆, 693 mg; vitamin D₃, 600,000; vitamin E, 5,000 mg; vitamin K, 196 mg; antioxidant, 100 mg.

³Diet 1 + anticoccidian (25,000 mg) and growth promoter (10,000 mg).

⁴35 and 42 d = diet 2 without anticoccidian.

⁵NuPro (Alltech do Brasil Agroindustrial Ltda. (Araucária, Paraná, Brazil).

⁶Bio-Mos (Alltech do Brasil Agroindustrial Ltda.).

42 d of age. Once serum and lacrimal secretions were taken, the samples were frozen at -20°C until they were submitted to analyses.

Serum antibody titers against NDV were measured by the hemagglutination inhibition test (HI) [43], and HI antibodies were then converted into $\log_2$. Immunoglobulin G titers against IBD were measured by the indirect enzyme-linked immunosorbent assay (ELISA) with a commercial kit [44]. Determination of IgA against the IBD found in lacrimal secretions was also measured by an indirect ELISA commercial kit [45] and a commercial goat anti-chicken IgA horse-radish peroxidase conjugate (chain specific) [46]. The HI titers were transformed into $\log_2$, and in the ELISA test, the optical density was

converted into titer values based on the calculation recommended by the kit manufacturer.

The methodology for IgA analysis was adapted to the commercial kit used to determine IgG levels in IBD, and only plates coated with the kit antigen were used. Substrate and reagents were the same as used in the indirect ELISA. For that purpose, the plates were incubated with a 1 M bicarbonate buffer solution (pH 7.4) and skimmed powdered milk (SPM) for 45 min at 37°C . The plates were then washed with PBS-Tween 20 (PBST; pH 7.4) and, after a PBST and SPM solution was added to the plate, samples were distributed in the solution. The plate was then placed in an oven at 37°C for 60 min. After this procedure, the plate was washed again with

PBST, followed by addition of PBST and SPM and finally the anti-IgA conjugate. The plate was placed back into the oven at 37°C for 120 min. After incubation, the plate was again washed with PBST, followed by the addition of a substrate solution containing *O*-phenylenediamine dihydrochloride, citrate buffer, and oxygenated water. After 15 min, 1 M HCl was added to stop the reaction. The reading was carried out at 490 nm with a microplate reader [47].

Statistical Analyses

Prior to statistical analyses, the authors checked for the presence of outliers and normality assumptions of Studentized errors (Cramér-von-Mises test) and for homogeneity of variances (Brown and Forsythe test). Analysis of variance was conducted with the GLM, a procedure of the SAS program [48]. Whenever significant differences were found, mean values were compared by Tukey's test (5%).

RESULTS AND DISCUSSION

The results for serum antibody titers (IgG) against NDV and IBD and lacrimal titers (IgA) in broilers supplemented with prebiotic and yeast extract in the prestarter diet and raised at different temperatures are presented in the Table 3.

No significant effects of the prebiotic and yeast extract on the analyzed parameters were observed; however, it is important to highlight that according to preliminary trials [20, 49], the addition of yeast extract to broiler prestarter diets promoted better feed conversion and better livability at 21 d of age.

Although there was no significant effect of prebiotic on the immune response, it is important to note that additives such as prebiotics with high mannose levels increase the protective antibody response and improve resistance to diseases while reducing the acute response [20, 50]. By direct action, we can assume that some specific prebiotics may reduce pathogen translocation in the intestine [51]. These compounds would bind to macrophage reception sites by recognizing specific sugars found in glucoproteins of the epithelial surface, triggering a cascading reaction that would eventually activate macrophages and release cytokines, thereby activating the acquired immune response [52].

Various experiments have been conducted to examine the effect of mannan oligosaccharides on humoral and cell immunity. Lilburn et al. [53] demonstrated that inclusion at 0.5 and 1 g/kg enhanced the serum antibody titer against sheep erythrocyte antigen administered 1 wk before evaluation when using a microtiter procedure. The stimulatory effect of mannan oligosaccharide on antibody titer was statistically significant 7 d after sensitization, and numerical differences persisted throughout the following 4 wk as titers waned. Inclusion of mannan oligosaccharide at 1 g/kg of diet enhanced both IgG and IgA serum antibody levels in turkey poult, and statistically significant increases in both Ig were detected at 7.5 wk of age [54].

Swanson et al. [55], who supplemented the diet of adult dogs with 2 g/d of prebiotics, found increased IgA levels in the ileum. However, when prebiotics were added to the prestarter diet in the present study (when the immune system was preparing to begin its activity), the humoral immune response was not affected; however, the response after inclusion must be followed up in later phases of the production cycle.

We found no studies in the literature in which yeast extract was used only in the starter diet, nor did we find studies that used the additive at different temperatures, with the supply of the products only in the prestarter. In the few existing studies, yeast extract was fed to broiler chickens during the whole cycle of production (1 to 42 d).

There was no systemic response to the 8-d NDV vaccination, whereas the IBD vaccination at 8 d decreased antibodies but increased them when injected on d 18. These results support interference caused by passive antibodies. Maternal protection is essential to protect the birds in their first days of life against challenges in the field, but it has now been proven that immunity also acts to interfere with the virus vaccine in vaccination success.

There was a significant interaction between brooding temperature and age at collection on the humoral immune response in all the parameters assessed (see Tables 4, 5, and 6). It is important to emphasize that the temperatures in this study were not repeated in other climate chambers or through repetition over time because these procedures are costly owing to the number

Table 3. Serum antibody titers (IgG) against Newcastle disease virus (NDV) and infectious bursal disease virus (IBD), and lacrimal titers (IgA) in broilers supplemented with prebiotic and yeast extract in the prestarter diet and raised at different temperatures

Factor	Antibody titer		
	NDV ¹ -IgG	IBD ² -IgG	IBD ² -IgA
Temperature (T)			
High	4.81 ^b	1,153.11	2.03
Control	5.29 ^{ab}	1,243.35	2.33
Low	5.52 ^a	1,118.32	3.03
Prebiotic (P)			
With	5.22	1,243.55	2.17
Without	5.19	1,101.69	2.22
Yeast extract (YE)			
With	5.16	1,195.32	2.21
Without	5.26	1,147.87	2.27
CV, %	35.28	49.92	41.00
Age at collection (A), d			
7	7.73 ^a	1,265.4 ^b	2.26 ^b
14	6.98 ^a	219.2 ^{cd}	1.59 ^c
21	3.84 ^{bc}	124.3 ^d	2.01 ^b
28	4.42 ^{bc}	457.9 ^c	1.95 ^b
35	3.57 ^c	1,345.8 ^b	4.48 ^a
42	4.61 ^b	3,389.3 ^a	3.04 ^a
CV, %	27.89	45.02	28.83 ³
Probability			
T	0.0447	NS	NS
P	NS	NS	NS
YE	NS	NS	NS
A	<0.0001	<0.0001	<0.0001
P × T	NS	NS	NS
YE × T	NS	NS	NS
YE × P	NS	NS	NS
T × P × YE	NS	NS	NS
A × YE	NS	NS	NS
A × T	0.0012	0.0032	0.0007
A × P	NS	NS	NS
A × T × P	NS	NS	NS
A × T × YE	NS	NS	NS
A × P × YE	NS	NS	0.0122
A × T × P × YE	NS	NS	NS

^{a-d}Mean values followed by the same letters in the columns do not differ according to Tukey's test ($P > 0.05$)

¹Antibody titers by the hemagglutination inhibition test ($\log_2$).

²Antibody titers by the enzyme-linked immunosorbent assay method.

³Transformed variable = $y^{-9.0988}$.

of animals with which we work. In this context, it would be good to note that several researchers conducted their field testing with the procedures used in this experiment.

Given the ability of broilers to respond with active immunity as of the second week of life, and given that innate immunity and maternal antibodies are active in previous stages [56], a plausible explanation for the differences in NDV-IgG at 7 d of age for high and low tem-

peratures (Table 2) would be that the transfer of IgG in the yolk sac membrane to the blood occurs until d 2 posthatch [57], when the yolk sac is absorbed. Environmental changes affect circulating hormones, such as T_3 and T_4 , which in turn may affect the speed of yolk sac absorption [1, 58]. In addition, the exposure of young birds to high ambient temperatures prior to primary antigenic changes inhibits the development of the primary immune response [59].

Table 4. Interaction of the effects of higher temperature on serum antibody titers (IgG) against Newcastle disease virus¹ in broilers supplemented with prebiotics and yeast extract in the prestarter fraction and raised at different temperatures

Age	Temperature			Probability
	High	Control	Low	
7	6.97 ^{a,B}	7.84 ^{a,AB}	8.37 ^{a,A}	0.0231
14	5.84 ^{ab,B}	7.56 ^{a,A}	7.53 ^{a,A}	0.0008
21	4.16 ^b	3.21 ^c	4.15 ^b	NS
28	3.94 ^b	4.31 ^{bc}	5.00 ^b	NS
35	3.40 ^b	3.19 ^c	4.13 ^b	NS
42	4.56 ^{b,AB}	5.34 ^{b,A}	3.94 ^{b,B}	0.0245
Probability	<0.0001	<0.0001	<0.0001	

^{a-c}Means in a column having no common superscript differ according to Tukey's test ($P < 0.05$).

^{A,B}Means in a row having no common superscript differ according to Tukey's test ($P < 0.05$).

¹Antibody titers by hemagglutination inhibition test ($\log_2$).

Stress influences the immune system function by stimulating the hypothalamic-pituitary-adrenal axis [60], which stimulates the secretion of corticosterone and adrenocorticotrophic hormone (ACTH). High corticosterone levels cause involution of lymphoid organs, such as the thymus, bursa, and spleen, and the redistribution of subpopulations of T lymphocytes [61, 62]. The bursa is an essential organ in the differentiation process of B lymphocytes and in the diversification of specificity in specific antibodies [63, 64], and the microenvironment of the thymus allows for the clonal formation of specific T-lymphocyte receptors, turning them into T-helper (CD4+) and T-cytotoxic (CD8+) lymphocytes [65].

The data collected contrast with those reported by Oba [66], who assessed the immune response to NDV in birds submitted to different temperatures and supplemented with chromium,

and found the lowest titer value for NDV at 7 d under cool temperatures.

At 42 d of age, antibody titers for NDV were the highest at control temperatures, whereas the lowest titers were found at low temperatures (Table 4). The antibody titers for NDV at high temperatures were not statistically different from those found at low and control temperatures. The decrease found at 42 d of age at low temperatures was probably related to the greater susceptibility of birds to low temperatures because of the stronger adrenal activity triggered by stress.

Overall, the study showed the influence of ambient temperature on the immune system at critical ages, that is, over the first 2 wk. In addition, the low temperature acted as an immunomodulator, improving the humoral immune response to NDV when compared with ther-

Table 5. Interaction of the effects of higher temperatures on serum antibody levels (IgG) against infectious bursal disease¹ in broilers supplemented with prebiotics and yeast extract in the prestarter phase and raised at different temperatures

Age	Temperature			Probability
	High	Control	Low	
7	1,675 ^{b,A}	1,186 ^{b,B}	934 ^{bc,C}	0.0004
14	241 ^c	226 ^c	149 ^c	NS
21	100 ^c	113 ^c	101 ^c	NS
28	247 ^c	492 ^{bc}	618 ^{bc}	NS
35	1,267 ^b	1,544 ^b	1,229 ^b	NS
42	3,143 ^{a,B}	3,649 ^{a,A}	3,376 ^{a,AB}	0.0273
Probability	<0.0001	<0.0001	<0.0001	

^{a-c}Means in a column having no common superscript differ according to Tukey's test ($P < 0.05$).

^{A-C}Means in a row having no common superscript differ according to Tukey's test ($P < 0.05$).

¹Antibody titers by the enzyme-linked immunosorbent assay test.

Table 6. Interaction of the effects of higher temperature on lacrimal antibody titers (IgA) against infectious bursal disease¹ in broilers supplemented with prebiotics and yeast extract in the prestarter fraction and raised at different temperatures

Age, d	Temperature			Probability
	High	Control	Low	
7	1.68 ^b	2.16 ^{ab}	1.85 ^{bc}	NS
14	1.31 ^{b,B}	2.14 ^{ab,A}	1.32 ^{c,B}	0.0007
21	1.98 ^{ab}	1.57 ^b	2.22 ^b	NS
28	1.80 ^b	1.81 ^{ab}	1.94 ^{bc}	NS
35	2.75 ^{a,B}	2.58 ^{a,B}	4.70 ^{a,A}	0.0161
42	2.90 ^a	2.36 ^a	3.32 ^a	NS
Probability	<0.0001	0.0001	<0.0001	

^{a-c}Means in a column having no common superscript differ according to Tukey's test ($P < 0.05$).

^{A,B}Means in a row having no common superscript differ according to Tukey's test ($P < 0.05$).

¹Antibody titers by the enzyme-linked immunosorbent assay test.

moneutral conditions, and these results support the findings of Henken et al. [34] and Oba [66].

The lower humoral immune response of broilers raised at low temperatures, compared with thermoneutral conditions, can be explained by an association between the stress on the hypothalamic-pituitary-adrenal axis [60] and the production of IL-1 (IL-1) by macrophages, stimulated by antigen. Interleukin-1 stimulates the hypothalamus, leukocytes, or both to produce the corticotropin-releasing factor, which stimulates the production of ACTH by the anterior pituitary, leukocytes, or both. In addition, corticotropin-releasing factor can directly increase lymphocyte activity in the spleen, causing ACTH to stimulate the production of corticosteroids, which in turn will redistribute secondary lymphocytes, such as to the spleen, to process the antigen and produce antibodies against the invading antigen [67].

The breakdown study with different temperature ranges for antibody titers against IBD showed a statistical difference among the 3 temperatures at 7 and 42 d of age (Table 3). At 7 d, the birds raised at low temperatures had lower antibody titers for IBD when compared with the birds raised at high and control temperatures. The findings are different from those shown by others [66], who assessed the immune response for IBD and found no statistical difference among these temperatures and ages.

There are genetic differences in the time required to transfer the breeder's antibodies to the egg by breeder immunization [67]. The percentage of eggs laid and the yolk weight are also im-

portant factors in the efficiency of IgG deposition in the egg [68], hence in the IgG available in the yolk subsequently to be transferred to the embryo. According to Shephard and Shek [69], cold stress suppresses lymphocyte proliferation, promotes a negative feedback of the immunological cascade, and reduces the number of natural killer cells and cytolytic activity.

The hypothalamus translates stress stimuli into neuroendocrine factors that can change the immune function [1, 70]. Under specific circumstances, corticosteroids may act as immunostimulants or immunomodulators, and they may regulate the development of both cellular and humoral immune responses, favoring and challenging the resistance to several pathogens [71]. During early response to stress, ACTH is released, leading to, among other physiological changes, increased blood lymphocyte levels to repair any possible physical damage and to fight any occasional aggressive agent [69]. This explains why the best maternal antibody values are found at high temperatures at 7 d of age.

At 42 d of age, birds raised at high temperatures had lower antibody titers for IBD when compared with birds raised at control temperatures. This can be explained by the greater sensitivity of the birds to heat stress at this age. This may have triggered the release of corticosterone, which in turn promoted the involution of lymphoid organs and, consequently, a lower humoral immune response to IBD.

On studying different ages for variable IgA, we found the only statistical difference at 14 and 35 d, when the birds raised at control and

low temperatures, respectively, had higher IgA values (Table 6). Despite the lack of statistical difference in IgA titer values in the lacrimal secretion of birds raised at low temperatures, these values tended to remain higher from 21 to 42 d of age when compared with other temperatures. There was a difference in the titer values of the birds at 14 d of age, indicating that the high and low temperatures affected the systemic response of the birds at this age.

CONCLUSIONS AND APPLICATIONS

1. The addition of yeast extract and prebiotic in the prestarter phase had no effect on the humoral immune response to NDV and IBD throughout the production cycle of the broilers.
2. The higher temperature increased the IgG values, because it particularly affected maternal antibodies, despite the immunomodulating effect caused by heat stress until the wk 2 of life.
3. Low temperatures favored higher and constant IgA levels in lacrimal secretion during the growth phase of the broilers.
4. Further studies are required to assess the potential benefit of low broiler brooding temperatures on mucosal protection.

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