Dietary arginine stimulates humoral and cell-mediated immunity in chickens vaccinated and challenged against hydropericardium syndrome virus

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ABSTRACT The effects of dietary supplement of arginine on protective humoral and cell-mediated immune responses of broiler chicks vaccinated and challenged against hydropericardium syndrome virus (HPSV) were investigated and compared with those of 2 reference drugs (cyclophosphamide and cyclosporine). Percentage ratios of lymphoid organs (bursa, spleen, and thymus) to BW, postvaccination and challenge serum antibody responses to HPSV, cutaneous basophil hypersensitivity reaction, peripheral lymphoproliferation, postchallenge detection of HPSV in the tissues of infected birds, and ability of chicks to resist virulent HPSV challenge were the parameters utilized to determine the effects of arginine on protective immune responses of chicks. A total of 600 chicks were used in this study. Arginine-supplemented chicks showed significant \( P < 0.05 \) stimulation of lymphoproliferation and cutaneous basophil hypersensitivity reactions compared with untreated control chicks. Similarly, significantly higher body and lymphoid organ weights were \( P < 0.05 \) recorded in arginine-supplemented chicks compared with untreated control chicks. The highest survival rate was recorded in arginine-supplemented HPSV-vaccinated chicks compared with immune-suppressed (cyclophosphamide- and cyclosporine-treated and HPSV-vaccinated chicks) and untreated unvaccinated control chicks after virulent HPSV challenges. Postchallenge tissue samples from arginine-supplemented and HPSV-vaccinated chicks yielded negligible HPSV detections by virus isolation in cell culture or PCR method, or both, compared with untreated control chicks. Thus, it was concluded that dietary supplementation of arginine had beneficial effects on humoral and cell-mediated immune responses of broiler chicks against HPSV.

Key words: arginine, antibody, cell-mediated immunity, broiler chick, hydropericardium syndrome virus

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INTRODUCTION

Hydropericardium syndrome (HPS) is a recently emerging immunosuppressive disease of poultry, which primarily affects 3- to 6-wk-old broiler chickens (Naeem et al., 1995; Ganesh and Raghavan, 2000; Balamurugan and Kataria, 2004, 2006; Schonewille et al., 2008). Hydropericardium syndrome has been reported in several countries including Mexico, Ecuador, Peru, Chile, Slovakia, Russia, India, Pakistan, Iraq, and Japan (Naeem et al., 1995; Mazaheri et al., 1998; Ganesh and Raghavan, 2000; Balamurugan and Kataria, 2004; Schonewille et al., 2008). The disease is characterized by sudden onset, accumulation of straw-colored fluid in the pericardium, and hepatitis with large, round basophilic intranuclear inclusion bodies in hepatic cells (Abdul-Aziz and Hasan, 1995; Naeem et al., 1995; Nakamura et al., 2003; Balamurugan and Kataria, 2004; Schonewille et al., 2008). The etiological agent of HPS is a serotype 4 fowl adenovirus, which is a nonenveloped, icosahedral, double-stranded DNA virus belonging to the adenovirus C species of the adenovirus genus in the family Adenoviridae (Abdul-Aziz and Hasan, 1995; Naeem et al., 1995; Nakamura et al., 2003; Balamurugan and Kataria, 2004; Schonewille et al., 2008). The mortality in HPS-affected broiler chicken flocks depends upon the virulence of the HPS virus (HPSV) and presence of other immunosuppressive factors in host birds and could reach 80% (Abdul-Aziz and Hasan, 1995; Naeem et al., 1995; Mazaheri et al., 1998; Ganesh and Raghavan, 2000; Balamurugan and Kataria, 2004; Schonewille et al., 2008).
Effective immunization against HPSV through the use of inactivated vaccines prepared from liver homogenates of infected chickens is the major practice employed to control this disease (Toro et al., 1999; Balamurugan and Kataria, 2004; Munir et al., 2007; Schonewille et al., 2008; Munir et al., 2009).

Several factors including immune status, type of infectious agent, and environmental, genetic, physiological, toxicological, and dietary factors may affect the 2 important functional aspects of the avian immune system (i.e., humoral and cell-mediated arms that form the basis for protective immune responses against viral and bacterial pathogens). There are several studies reporting the effects of dietary arginine on various aspects of immune functions in animals and humans (Barbul et al., 1980, 1981; Daly et al., 1988; Reynolds et al., 1990; Park et al., 1991; Kirk et al., 1992; Wightham et al., 1992; Ochoa et al., 2001). The immunomodulatory effects of arginine in animals include increased nitric oxide production by macrophages (Hibbs et al., 1987; Amber et al., 1991; Tsai et al., 2002), improved thymic weight and function (Barbul et al., 1980), enhanced lymphocyte response to mitogens such as concanavalin A and phytohemagglutinin (PHA; Ochoa et al., 2001), improved immunity against tumors (Capuano et al., 2009), enhanced wound healing (Wu et al., 2008), and stimulatory effects either on the production or function of cytokines and other cells of the immune system (Ochoa et al., 2001; Wu et al., 2008). Arginine is also one of the factors required for differentiation and release of B lymphocytes from the bone marrow (Park et al., 1991; de Jonge et al., 2002).

Chickens are unable to synthesize arginine due to the incomplete urea cycle they possess (Cuca and Jensen, 1990; Kidd et al., 2001). Two recent reports have indicated that arginine significantly improves the capacity of chickens to surmount immunosuppression induced by vaccinal strains of infectious bursal disease virus (Tayade et al., 2006a,b). However, research evaluating effects of arginine on immune function responsiveness of chickens to other infectious agents is still sparse, especially if this amino acid is to be used as a common immunomodulator in poultry flocks. Currently, there are no objective data available on the immunomodulatory effects of arginine against HPSV in broiler chickens. The present study evaluates arginine as an immunomodulator on protective responses against HPSV. To our knowledge, this report is the first of its kind that assesses the effects of dietary arginine on the protective humoral and cellular immune responses in broiler chickens vaccinated and challenged against HPSV.

MATERIALS AND METHODS

Chicks

Day-old (Hubbard × Hubbard) broiler chicks obtained from a local hatchery were used in this study. Chicks in all experimental groups were raised in separate floor pens in the same room under similar management conditions with feed and water ad libitum.

Vaccine and Virulent HPSV

An inactivated oil emulsion vaccine against HPS prepared as described previously (Roy et al., 1999; Munir et al., 2007, 2009) was used in this study. Challenge trials were conducted with virulent HPSV (vHPSV) characterized in our previous study (Munir et al., 2007). The identity of vHPSV isolate was confirmed by agar gel precipitation (AGP) test using HPSV-specific antiserum as described elsewhere (Naem et al., 1995). The antiserum raised elsewhere (Munir et al., 2007) was used during this study. The identity of the vHPSV was further confirmed by recording the HPS-specific gross lesions in ten 3-wk-old susceptible broiler chickens after infection with the virulent virus. The 50% tissue culture infectious dose of the HPSV was determined using the method of Reed and Muench (1938).

Viral DNA Amplification and Detection

The PCR method described previously (Ganesh et al., 2002) was used to amplify HPSV-specific DNA from tissue sections collected from vHPSV-infected and uninfected control chicks. The specificity of PCR products was checked by visualization under UV light after gel electrophoresis and staining with ethidium bromide.

Drugs

Cyclophosphamide (CYP; CYP 2H-1, 3, 2-oxasophosphasine) and cyclosporine (CYS) were used as reference drugs to induce immunosuppression. Cyclophosphamide is known to cause humoral suppression, whereas CYS is known for its T-cell-specific immunosuppressive effects.
**AGP Test**

Two-fold dilutions of postvaccination and postchallenge sera of chicks from all groups were prepared. The precipitating antibodies to HPSV in 1:2, 1:4, 1:8, and 1:16 dilutions of sera were detected using the AGP test, which was performed as described previously (Naeem et al., 1995; Munir et al., 2007).

**Peripheral Lymphocyte Proliferation Assay**

This assay was performed according to procedure described elsewhere (Sun et al., 2006) with minor modifications. Briefly, 5 mL of blood samples collected directly from the heart of chicks in aseptic tubes containing sodium heparin and mixed with an equal volume of Hank’s solution were layered on lymphocyte separation medium. After centrifugation at 1,000 × g for 20 min, the lymphocytes were collected and washed 2 times with RPMI-1640 medium without any fetal bovine serum (FBS) supplement. Cells were adjusted to 4 × 10⁶ per milliliter in RPMI-1640 medium containing 10% FBS, streptomycin (100 IU per mL), and benzyl penicillin (100 IU per mL). Each sample was seeded into 8 wells of a 96-well culture plate with each well receiving 80 µL of cell suspension. Each of the first 4 of 8 wells received 20 µL of PHA, a T-cell mitogen, whereas each of the remaining 4 wells received 20 µL of RPMI-1460 medium supplemented with antibiotics and FBS. The plates were incubated at 38°C in a CO₂ incubator at 5% CO₂ tension for 48 h. After incubation, 20 µL of 4,5-dimethyl-thiazole-2, 5-diphenyl tetrazolium bromide (MTT) was added to each well and plates were reincubated for 4 h. After incubation, plates were centrifuged at 1,000 × g for 10 min at room temperature (22°C). Supernatant was discarded, and 100 µL of dimethyl sulfoxide per well were added to extract the formazan. Optical density (OD) of cells in each well was measured at a wavelength of 570 nm.

**Cutaneous Basophil Hypersensitivity Response**

The cutaneous basophil hypersensitivity (CBH) responses of chicks in various groups were assessed according to a procedure described previously (Corrier and DeLoach, 1990; Boa-Amponsem et al., 2000). Briefly, each chick was injected intradermally in the toe web between the third and fourth digits of the right foot with 100 µg of PHA in 100 µL of PBS. Each chick was sham-injected with 100 µL of PBS in the corresponding toe web of left foot, which served as a control. The toe web thickness was measured before and 24 h after the PHA or PBS injection with CBH response being equal to the difference in the toe web thickness between the right foot and left foot, where toe web thickness of the right foot = post-PHA injection thickness – pre-PHA injection thickness and toe web thickness of the left foot = post-PBS injection thickness – pre-PBS injection thickness.

**Experimental Design**

A flowchart depicting experimental design is presented in Figure 1. A total of 600 one-day-old chicks were used in this study. Chicks, upon their arrival, were color-marked and randomly divided into 6 groups of 100 chicks per treatment. Chicks in group 1 received feed supplemented with 2% arginine throughout the experimental period. Chicks in group 2 and other groups were fed rations without any arginine supplement. Chicks in group 3, during the first 5 consecutive days of their life, were injected s.c. daily in their abdominal region with a solution that contained 3.0 mg of CYP, 12.5 IU of penicillin, and 0.0125 mg of streptomycin per milliliter. Chicks in group 4 were injected i.m. with CYS solution in olive oil at a dose of 100 mg/kg of BW every 3 d for the first 5 wk of their life. On d 14 of age, an oil-based inactivated HPS vaccine was injected s.c. in the neck regions of chicks from group 1, 2, 3, and 4. Chicks in group 5 and 6 served as untreated unvaccinated control and were injected with sterile PBS solution. To evaluate HPSV-specific antibody responses, blood samples were collected on d 0 (immediately after vaccination) and thereafter weekly up to 5 wk postvaccination every time from 15 randomly selected chicks in each of 6 groups for serum analysis by the AGP test. On d 7, 14, 21, and 35 after vaccination, blood samples (5 mL from each chick) were also collected directly from the heart of 5 chicks in group 1, 2, 3, and 4 in heparin-containing sterile tubes for assay of peripheral lymphoproliferation by the MTT method. On postvaccination d 21, five randomly selected chicks in each of 6 groups were tested for their CBH responses for an in vivo assay of cell-mediated immunity. On d 28 of age, 45 chicks from each group of 1, 2, 3, 4, and 5 were removed and transferred to pens in a separate room for the challenge trial with vHPSV; each chick was challenged i.m. with 0.5 mL (10⁶ 50% tissue culture infectious dose) of vHPSV suspension. Of 45 chicks, 30 chicks were used for tissue sample collection for virus detection by cell culture and PCR methods; every 5 of these chicks were killed on post-vHPSV challenge d 0, 3, 5, 7, 9, and 11 to collect their liver and spleen. Chicks in group 6 were sham-challenged with sterile PBS. The remaining 15 of 45 challenged chicks from each group were kept in a different pen and were monitored for daily mortality up to 11 d postvirulent challenge; postmortem examination was also performed on dead birds to record lesions. In addition, blood samples of chicks that survived vHPSV challenge up to 11 d were also collected to determine the HPSV-specific antibodies in postchallenge sera using the AGP test. On d 42 of age, 10 randomly selected chicks (of 55 chicks that stayed in their original pens) in each of 6 groups were weighed, killed, and their bursa, spleen, and thymus were removed and weighed to assess lymphoid organ:BW ratios.
Statistics

The mean and 95% confidence intervals of BW and percentage ratios of organs to body weights of chicks in various groups were estimated as described previously (Giambrone and Closser, 1990; Shivachandra et al., 2003). Statistical significance was set at $P < 0.05$. Logistic regression was used to determine the significant effects of different treatments on the antibody response to HPSV, and also on postchallenge HPSV detection by virus isolation or PCR in tissue samples, or both. The significant differences among mean OD values of chickens in different experimental groups were determined by the GLM (Christensen, 1998). The significant differences among mean of CBH responses in various groups were determined by the GLM. Bonferroni procedure was used to adjust for multiple group comparison (Christensen, 1998). Minitab version 15.0 (Minitab Inc., State College, PA) was used to perform all of the statistical analyses.

RESULTS

Body and Lymphoid Organ Weights

The mean body and lymphoid organ weights together with percentage ratios of these organs to BW for chicks in various groups are summarized in Table 1. Overall,
there was significant difference among the mean BW of chicks in various groups. Body weight at 42 d of age in the arginine-supplemented and HPS-vaccinated chicks (group 1) was significantly higher \((P < 0.05)\) than all of the other groups (Table 1). The BW was significantly decreased \((P < 0.05)\) from group 1 but significantly higher \((P < 0.05)\) than group 4 but significantly lower \((P < 0.05)\) than group 1 and 2.

The OD measurements of the MTT assay are a function of lymphoproliferation. The mean OD values of groups on different days after HPS vaccination are shown in Figure 3. The OD values of arginine-supplemented and HPS-vaccinated chicks (group 1) were significantly higher \((P < 0.05)\) than any other HPS-vaccinated group. On nearly all tested days, OD values of arginine-supplemented and HPS-vaccinated chicks (group 1) were significantly higher \((P < 0.05)\) than group 4 but significantly lower \((P < 0.05)\) than group 1 and 2.

The results on HPSV-specific antibody responses in postvaccination sera tested by AGP are presented in Table 2. The 1:2, 1:4, 1:8, and 1:16 dilutions of each serum were analyzed. The highest number of sera in group 1 (arginine-supplemented and HPS-vaccinated) tested positive for HPSV-specific antibodies compared

**Postvaccination Serum Antibody and Cell-Mediated Immune Responses**

The CBH responses of chicks in various groups at 35 d of age are depicted in Figure 2. The arginine-supplemented and HPS-vaccinated chicks (group 1) had significantly higher \((P < 0.05)\) CBH responses than all other groups. Similarly, CBH responses of HPS-vaccinated control-fed chicks (group 2) were significantly decreased from group 1 but significantly higher \((P < 0.05)\) than the CBH responses from CYP- and CYS-treated and HPS-vaccinated chicks (group 3 and 4, respectively). The untreated unvaccinated control (group 5) and untreated unvaccinated challenge control birds (group 6) had CBH responses that were significantly higher \((P < 0.05)\) than group 4 but significantly lower \((P < 0.05)\) than group 1 and 2.

![Figure 2. Mean cutaneous basophil hypersensitivity (CBH) responses of chicks in various groups following postphytohemagglutinin challenge. The error bars indicate SE. Chicks in group 1, 3, and 4 were hydropericardium syndrome-vaccinated and received arginine supplement, cyclophosphamide, and cyclosporine treatments, respectively, whereas those in group 5 and 6 served as untreated, unvaccinated control. Chicks in group 2 served as untreated vaccinated control. Groups carrying different letters are significantly different from each other \((P < 0.05)\), whereas those carrying the same letters are not significantly different from each other \((P > 0.05)\).](https://academic.oup.com/ps/article-abstract/88/8/1629/1537440)
with sera of other groups; on d 35 postvaccination, 9/10 sera tested positive at the highest (average) dilution (i.e., 1:16) compared with 6/10 positive sera of group 2 (untreated vaccinated), which tested at an average dilution of 13.33. As expected, none of the sera in unvaccinated group 5 (untreated unvaccinated control) and 6 (untreated unvaccinated challenge control) showed any positive reaction for the presence of HPSV-specific antibodies. Compared with group 1 (arginine-supplemented and HPS-vaccinated), few sera of CYP- and CYS-treated and HPS-vaccinated chicks (group 3 and 4, respectively) tested positive for HPSV-specific antibodies by the AGP test on different days postvaccination (Table 2).

### Clinical Signs, Survivability, Lesions, Virus Detections, and Antibody Responses After HPSV Challenge

The highest survival rate (100%) was observed in group 1, whereas the lowest survivability was recorded in group 5 (untreated unvaccinated chicks 6) after challenge with vHPSV treatment (Table 3). Chicks treated with CYP (group 3) and CYS (group 4) showed the lowest postchallenge survival rates among the HPS-vaccinated groups. The maximum mortality was recorded between d 3 and 6 after infection with vHPSV. Depression and ruffled feathers were the only clinical signs observed in chicks that died after infection with vHPSV. The gross lesions recorded in these chicks were lung congestion, presence of straw-colored fluid in the pericardial sac, enlarged and mottled liver with necrotic foci, and nephritis (Figure 4).

The results of HPSV detection in post-vHPSV challenge tissues by cell culture and PCR methods are shown in Table 4. Only 1 sample from group 1 (arginine-supplemented and vaccinated) tested positive for HPSV-specific DNA by the PCR assay. Virus could not be isolated from any of the tissue samples in group 1 (arginine-supplemented and vaccinated). As expected, higher numbers of virus isolations/detections were made from tissues of group 3 (CYP-treated and vaccinated) and 4 (CYS-treated) as well as group 5 (untreated unvaccinated control chicks) after vHPSV challenge, and most of these virus isolations were made within 3 to 7 d of vHPSV challenge (Table 4). The maximum number of postchallenge sera (14/15) from group 1 (arginine-supplemented and vaccinated) tested positive for HPSV-associated antibodies by the AGP test followed by group 2 (untreated vaccinated; 10/13), group 4 (CYS-treated and vaccinated; 3/7), and group 3 (CYP-treated and vaccinated; 1/6). None of the sera from group 6 (untreated unvaccinated challenge control birds), which did not receive vHPSV challenge, tested positive for precipitating antibodies to HPSV. All posi-

### Table 2. Postvaccination serum antibody response against hydropericardium syndrome virus (HPSV) in chickens of various experimental groups

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<tr>
<th>PVD</th>
<th>Group 1</th>
<th>Group 2</th>
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1Chicks in group 1, 2, 3, and 4 were vaccinated on d 14 of age with an oil-based inactivated hydropericardium syndrome vaccine injection s.c. in their neck regions; chicks in group 5 and 6 were injected with sterile PBS solution; see Table 1 for treatments given to each of the 6 groups.

2PVD = postvaccination day.

3Day 0 = immediately after vaccination.

4Numbers represent the average of serum dilutions that tested positive for HPSV-specific antibodies.

5Numbers in parentheses represent positives out of total sera tested by the agar gel precipitation test.

Figure 3. Mean optical density (OD) values of hydropericardium syndrome (HPS)-vaccinated groups on different days. Mean OD measurements are a function of lymphoproliferation of birds in each group. Group 1, 3, and 4 were HPS-vaccinated and received arginine supplement, cyclophosphamide, and cyclosporine, respectively, whereas group 2 served as vaccinated control-fed chicks. The mean OD value of group 1 was significantly higher (P < 0.05) than any other group. See text for detail.
tive serum samples of group 1 (arginine-supplemented and vaccinated) and 2 (untreated vaccinated) showed positive reaction at 1:16 dilution. None of the chicks in group 5 (untreated unvaccinated control) survived the vHPSV challenge up to 11 d for serum sample collection.

**DISCUSSION**

The main objective of this study was to evaluate arginine as an immunoregulator of the protective humoral and cell-mediated immune (CMI) responses against HPSV. The criteria used for evaluating the effects of arginine on protective responses of birds against HPSV were those suggested or utilized previously for assessing the immunomodulatory effects of a drug or chemical (Dohms and Saif, 1984; Muneer et al., 1988; Wu et al., 2000; Kim et al., 2004; Munir et al., 2007, 2009).

We used 2% arginine supplement in feed because previous reports (Tayade et al., 2006a,b) have suggested that this level of arginine supplement is safe and does not cause any side effects to birds. The results that dietary arginine supplement had positive effects on growth of immunoregulatory organs are similar to those reported by Tayade et al. (2006a), who reported an increase in bursa:BW and spleen:BW ratios of chickens that received dietary arginine supplement. A growth response of over 20% was observed in arginine-supplemented birds (group 1) compared with untreated unvaccinated birds (group 5 or 6; Table 1). Similarly, BW of the 42-d-old chickens (Table 1) appear to be low for broiler chicks of this age. It is possible that the basal diet (we used a commercial feed) offered to birds in this study was either imbalanced for amino acid ratios or deficient in certain nutrients such as arginine; alternatively, although less likely, variation in ambient temperature might have affected the feed consumption or metabolism of birds during the course of this study. Chamruspollert et al. (2004) have reported that the environmental temperature and imbalance of the ratios of amino acids such as arginine, methionine, or lysine in the diet could affect the BW gain of broiler chickens. The findings that overall the highest numbers of post-vaccination (and postchallenge)-positive sera for HPSV-associated antibodies and significantly higher OD and CBH values were observed in group 1 (arginine-supplemented and vaccinated) than any other group (Table 2) are concurrent with those of Tayade et al. (2006a,b), who also reported significantly higher ELISA antibody titers and enhanced lymphoproliferation and hypersensitivity reactions in arginine-supplemented chicks, who received infectious bursal disease virus vaccination. The higher protection (100%) observed in group 1 (arginine-supplemented and HPS-vaccinated; Table 3) is also harmonious with observations of Tayade et al. (2006a,b). The findings that the lowest survival rates

| Table 3. Mortality in experimental groups on different days after virulent hydropericardium syndrome virus (vHPSV) challenge
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1Chicks from group 1, 2, 3, 4, and 5 were challenged i.v. with 0.5 mL (10^6.0 50% tissue culture infectious dose) of vHPSV suspension.
2Chickens in experimental group 5 served as unvaccinated, untreated positive control for challenged trial; see Table 1 for treatments given to each of the 6 groups.
3Chicks in group 6 were sham-challenged with sterile PBS and served as negative (untreated, unvaccinated, and unchallenged) control for virulent challenge trial.
were recorded in group 3 (CYP-treated and HPS-vaccinated) and 4 (CYS-treated and HPS-vaccinated; 40 and 46.7%, respectively) are harmonious with previous reports (Munir et al., 2007, 2009). The further finding that most of the mortality due to vHPSV infection occurred in birds within 3 to 6 d of virulent challenge is also consistent with previous reports (Munir et al., 2007, 2009). The gross lesions recorded in birds after vHPSV challenge (Figure 3) are similar to those reported previously (Naeem et al., 1995; Munir et al., 2007, 2009). The results that HPSV or its specific DNA could not be detected in nearly all of the post-vHPSV challenge tissues of birds in group 1 are also similar to those reported previously (Munir et al., 2007, 2009). Attempts to measure the significant effects of various treatments on the postvaccination serum antibody responses and on virus isolation/detection were not successful when logistic regression was used. This was due to the absence or low numbers, or both, of positive samples in group 3 (CYP-treated and vaccinated), 4 (CYS-treated and vaccinated), 5 (untreated unvaccinated control), and 6 (untreated unvaccinated challenge control; Table 2 and 4); therefore, the data in Table 2 and 4 could not be analyzed using the logistic regression.

From the results of this study, an association could be established between the post-vHPSV challenge survival rates, numbers of postvaccination and postchallenge sera showing positive reactions for precipitating antibodies to HPSV, levels of OD values and CBH responses, and numbers of HPS viral isolations or specific DNA detection after challenge of chicks with vHPSV. The correlations between the detection of HPSV from tissues and post-vHPSV challenge mortalities in birds are similar to those reported previously (Naeem et al., 1995; Munir et al., 2007, 2009). It is not known whether the humoral or CMI or both arms of the specific immune system play a role in controlling the spread of HPSV infection in the host birds, and if both components play a role in protection, which of these components is more vital in affording the protection against HPSV. From the results of this study, it could be speculated that probably both arms of the immune system play a role in protection against HPSV infection as evident by the increased mortality and higher numbers of HPSV isolation/detection observed in CYP-treated (humoral immune-suppressed) as well as CYS-treated (cell-mediated immune-depressed) birds (Tables 2, 3, and 4; Figures 1, 2, 3, and 4). It has also been reported that HPSV causes immunosuppression by damaging lymphoid tissues of birds (Balamurugan and Kataria, 2006); this virus is known to cause reduction in both antibody-mediated and possibly CMI responses of broiler chickens against Newcastle disease virus (Naeem et al., 1995; Balamurugan and Kataria, 2006). Arginine supplementation could prove crucial in overcoming the immunosuppressive effects of HPSV in chickens because arginine helps in the differentiation of pro-B cell to pre-B cells and also in the release of these cells from bone marrow (Park et al., 1991; de Jonge et al., 2002). The depressed lymphoproliferation responses indicated by significantly lower OD values and CBH reactions in group 3 (CYP-treated and vaccinated) and 4 (CYS-treated and vaccinated) and significantly elevated lymphoproliferation responses demonstrated by significantly higher OD values and CBH responses in group 1 (arginine-supplemented and vaccinated) imply that arginine probably stimulated the T lymphocyte functions against HPSV. The lack of HPS viral isolations or viral-specific DNA detections from the tissue samples and negligible mortality after challenge of arginine-supplemented birds with vHPSV also implies that suppressive and replicating effects of HPSV were more rapidly cleared in arginine-supplemented birds than in other groups. It has been suggested that arginine acts on different components of the immune system (Ochoa et al., 2001; Wu et al., 2008). The profound effects on cell-mediated immunity (increased OD values and CBH responses) of arginine-supplemented birds probably occurred via proliferation response of T lymphocytes to PHA through the release of interleukin-2 because levels...
of interleukin-2 and proliferation of cytotoxic T lymphocytes have been shown to increase in a dose-dependent manner (Öchoa et al., 2001; Tayade et al., 2006a; Wu et al., 2008). Arginine has also been shown to act as a potent modulator of macrophage-mediated phagocytosis through arginine and nitric oxide synthase pathways (Hibbs et al., 1987; Amber et al., 1991; Tsai et al., 2002). Macrophages are considered central cells in specific and nonspecific immunity and are also capable of secreting potent cytokines including IL-1 and tumor necrosis factor-α (Hibbs et al., 1987; Amber et al., 1991; Tsai et al., 2002; Tayade et al., 2006a, b); CBH responses in toe webs of chickens, after inoculation of PHA, in arginine-supplemented and HPSV-vaccinated birds, were clearly elevated.

In summary, this study demonstrates for the first time that dietary supplementation of arginine enhances HPSV-specific humoral and CMI responses as well as provides complete protection against vHPSV challenge in HPSV-vaccinated broiler chickens. Thus, arginine could act as a valuable immunoregulator not only against HPSV but also against other poultry pathogens. However, the immunomodulatory effects of arginine require further evaluation under field conditions, where HPSV challenge is not only of diverse nature and variable magnitude but the presence of other field agents or factors could cause varying degrees of immunosuppression in birds.

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