INTRODUCTION

In the developed countries where poultry is commercially raised, poor response to vaccination is frequently observed and, in most instances, the failure to respond adequately is the result of immune suppression (Jackwood, 1991). Analysis of HI titers for Newcastle disease virus in vaccinated commercial broilers in Croatia, Slovenia and Bosna-Herzegovina for the decade preceding the current war revealed significant immune suppression (Ragland et al., unpublished observations). Infection with several viruses may result in immune suppression but with the exception of chicken anemia virus, infection usually is prevented by vaccination of parent flocks. Various chemical pollutants and toxins, especially mycotoxins, now may play a major role in immune suppression. Since many of these chemicals, especially the mycotoxins, cannot be removed from feed stocks, methods of preventing their imunosuppressive effects are needed.
need to be developed. Immune stimulants that can be added to the feed or water are obvious candidates. A feasible immune stimulant should be easily applied, inexpensive and not requiring a withdrawal period prior to slaughter.

Studies conducted by Bakalli and colleagues in Prishtina to evaluate toxic effects of heavy metals included experiments with broiler breeders fed 100 ppm lead in the diet. An unexpected finding was higher levels of HI titers for Newcastle disease in vaccinated hens exposed to lead than in unexposed hens (Domi et al., 1991). The present study is an extension of these investigations in broilers fed two levels of the metal calcium.

MATERIALS AND METHODS

Male chicks (Peterson x Arbor Acres) from a commercial hatchery were kept in battery cages with wire floors for 42 days in rooms supplied with filtered air under positive pressure to maintain them free of adventitious virus infection. They consumed feed and water ad libitum. Eight diets in a 2 x 4 factorial arrangement of 2 levels of calcium (0.65 and 1.30%), and 4 levels of lead (0, 1, 10 and 100 ppm from PbSO₄) were randomly assigned to three pens each. The low level of calcium results in maximum growth rate but low bone ash and a high incidence of leg problems, and the high level of calcium results in maximum growth rate with high bone ash and a low incidence of leg problems. The basal diets contained corn, soybean meal, poultry fat, vitamins and minerals. Chickens were vaccinated at 10 and 18 days of age for infectious bursal disease (IBD-BLENT™, CEVA Laboratories, Overland Park, Kansas) and at 21 days of age for Newcastle disease (Clonevac- 30®, Intervet, Millsboro, Delaware). Serum samples were collected at 1, 9, 18 and 30 days of age from at least 10 chickens in each group and assayed by enzyme-linked immunosorbent assay for antibody to the two diseases using commercial reagents (IDEXX Laboratories, Westbrook, Maine) to ascertain the immune status from maternal immunity and that the chickens were responding to vaccination. Antibody titers for the two vaccines were measured in serum samples collected from all chickens at 42 days of age. Body weights and bursal weights were used to calculate relative weights. Areas of 25 contiguous bursal follicles and their medullae were measured by morphometric analysis of tissue sections with Integrated Image Analysis System (Southern Micro Instrument, Atlanta, Georgia) coupled with an Olympus BX-TH microscope and a Zenith Systems microcomputer. The areas of their cortices were calculated by difference.

Statistical inference was determined by ANOVA using the method of least squares (SAS Institute, Cary, North Carolina).

RESULTS AND DISCUSSION

In all three experiments, antibody titers to Newcastle disease virus were greater in chickens fed 1.3 than 0.65% calcium (Tables 1, 4 and 6). Dietary lead increased titers to Newcastle disease virus in one experiment (Table 1).

Although the main effect means were not different with lead in the other two experiments (Tables 4 and 6), several group means, especially at 10 ppm lead, were significantly higher than chickens not fed lead, suggesting that lead may indeed interact positively with calcium.

Titers to infectious bursal disease virus in chickens fed 1.3% calcium were less in one experiment (Table 1) and unaffected in another experiment (Table 5). Low level of lead increased titers to infectious bursal disease virus in one experiment (Table 1) and had no effect in another experiment (Table 5).

Relative bursal weights were increased by dietary lead but not by increased calcium (Table 2). Bursal follicles were larger in birds fed 1.3 than 0.65% calcium, increase in medullary area contributing more increase in cortical area (Table 3). Dietary lead also contributed to greater follicular size. Since bursal lymphocytes migrate to the medulla as they undergo differentiation, this suggests that calcium, and possibly lead, may be a signal to immature cortical lymphocytes to undergo more rapid differentiation or maturation. More rapid differentiation of bursal lymphocytes would be consistent with enhanced immune responsiveness.

These effects on immune responsiveness may be mediated through an effect on a protein kinase C (PKC), which is activated by calcium, and the increased activity of PKC is associated with activation of immune cells (Hengel, 1991). Metals can affect PKC and adenyl cyclase transduction systems (Lawrence, 1985). Reports show that lead can stimulate brain PKC at picomolar concentrations whereas nanomolar concentrations of calcium are required to activate PKC (Markovac and Goldstein, 1988).

The effects of lead on immunity are still unclear. Reports in the literature vary among and within several species, suppression being observed more often than enhancement (Descotes et al., 1990; Kimber, 1990). Most of the studies have been done at high levels often lasting longer than our experiments. Nevertheless, our
data indicate a positive interaction between lead and calcium may exist, depending on the relative concentrations. The body of evidence suggests that increased calcium in the diet enhances the immune response to some but not all viral vaccines.

Although low levels of lead may have an additive effect, lead supplementation cannot be recommended. Calcium, on the other hand, would be an ideal immune stimulant if further studies with other antigens reveal similar results.

Table 1 - Influence of dietary lead and calcium on antibody titers for Infectious Bursal Disease (IBD) and Newcastle Disease (NCD) in broiler chickens (Experiment 1).

<table>
<thead>
<tr>
<th>Dietary lead (ppm) Olov u hran</th>
<th>Dietary Calcium (%) Kalcij u hran</th>
<th>0.65</th>
<th>1.3.</th>
<th>Mean ± S.E. Prosjek ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (n) Prosjek (n)</td>
<td>Mean (n) Prosjek (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 IBD</td>
<td>5495 (30) 1323 (23)</td>
<td>3685a ± 605</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 NCD</td>
<td>8604 (30) 16350 (23)</td>
<td>11965b ± 2861</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 IBD</td>
<td>8441 (29) 2224 (23)</td>
<td>5691a ± 1222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 NCD</td>
<td>11866 (29) 26940 (23)</td>
<td>14110a ± 2784</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 IBD</td>
<td>3491 (29) 2365 (22)</td>
<td>3005b ± 529</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 NCD</td>
<td>12083 (29) 19321 (22)</td>
<td>15205c ± 3322</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 IBD</td>
<td>3535 (30) 2630 (22)</td>
<td>3152b ± 647</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 NCD</td>
<td>10219 (30) 28060 (22)</td>
<td>17767c ± 4893</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE1 Prosjek ± SE1</td>
<td>IBD 5228a ± 639</td>
<td>2128b ± 335</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCD 10671a ± 1523</td>
<td>20089b ± 3518</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Main effect means with different superscripts are significantly different, p ≤ 0.05

Table 2 - Influence of dietary lead and calcium on the body weight and relative bursa weight of 42-day old broiler chickens (experiment 1)

<table>
<thead>
<tr>
<th>Dietary lead (ppm) Olov u hran</th>
<th>Dietary Calcium (%) Kalcij u hran (%)</th>
<th>0.65</th>
<th>1.3.</th>
<th>Mean ± S.E. Prosjek ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (n) Prosjek (n)</td>
<td>Mean (n) Prosjek (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Weight gain (kg) Težinski prirast (kg)</td>
<td>2.067 (3) 1.977 (3)</td>
<td>1.992 ± 0.014a</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Bursa weight (%) Težina burze (%)</td>
<td>1.188 (10) 1.177 (10)</td>
<td>1.152 ± 0.014b</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Weight gain (kg) Težinski prirast (kg)</td>
<td>1.920 (3) 1.990 (3)</td>
<td>1.965 ± 0.021a</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Bursa weight (%) Težina burze (%)</td>
<td>2.237 (10) 1.209 (10)</td>
<td>2.224 ± 0.015a</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Weight gain (kg) Težinski prirast (kg)</td>
<td>1.920 (3) 1.893 (3)</td>
<td>1.970 ± 0.016bc</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Bursa weight (%) Težina burze (%)</td>
<td>2.229 (10) 1.257 (10)</td>
<td>2.43 ± 0.02a</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Weight gain (kg) Težinski prirast (kg)</td>
<td>1.893 (3) 1.857 (3)</td>
<td>1.875 ± 0.014a</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Bursa weight (%) Težina burze (%)</td>
<td>2.228 (10) 2.260 (10)</td>
<td>2.24 ± 0.02a</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE1 Prosjek ± SE1</td>
<td>Weight gain (kg) Težinski prirast (kg)</td>
<td>1.905 ± 0.016</td>
<td>1.929 ± 0.019</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bursa weight (%) Težina burze (%)</td>
<td>0.220 ± 0.011</td>
<td>0.210 ± 0.016</td>
<td></td>
</tr>
</tbody>
</table>

1 Main effect means with different superscripts are significantly different, p ≤ 0.05
CONCLUSIONS

Antibody response to Newcastle disease vaccine but not infectious bursal disease vaccine is greater in chickens fed 1.3 than 0.65% calcium. Low level of dietary lead may also increase antibody responses. Relative bursal weight is increased by dietary lead. Dietary calcium and lead increase size of bursal follicles, mostly by increase in medullary size.

ACKNOWLEDGMENTS

This research was supported by funds from the Veterinary Medical Experiment Station and the Agricultural Experiment Station, University of Georgia, the Soviet and East European Affairs, office of the National Research Council (R. I. Bakalli), and a United States Information Agency scholarship awarded to R. Novak by the Baltic/East Central Europe Assistance Awards Program administered by NAFSA:Association of International Educators.

REFERENCES


Sažetak

Pilići mužjaci (Peterson i Arbor Acres) nabavljeni su u komercijalnom uzgajalištu i hranjeni smjesom što je sadržavala 0; 1; 10 i 100 ppm olova na dvije razine kalcija (0.65 i 1.30%). Za vrijeme držanja pilića nije došlo do slučajnih zaraza te su bili cijepljeni protiv zarazne bursalne bolesti u dobi od 10 i 18 dana i protiv Newcastle bolesti u dobi od 21 dan. Titri antijela za Newcastle bolest mjereni imunosorbenim uzorkom vezanim enzymom bio je znatno veći u šestom tjednu u skupinama hranjenim visokim razinama kalija. Niske razine olova (0; 1 i 10 ppm) znatno su porasle a najviši (100 ppm) su razine potisnule titere. Dodatni su bili stimulativni učinci kalija i olova. Titri za virus zarazne bursalne bolesti nisu se značajno izmijenili. Relativne bursalne težine povećale su se i smanjivale u skladu s titrima antijela za virus Newcastle bolesti. Morfometrička analiza presjeka bursalnog tkiva otkrila je da su se područja bursalnih folikula, kortikula i medula povećali u skupinama u kojima su se povećale relativne bursalne težine.

hrvatska gospodarska komora
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Zajednica strukovnih grupa industrije stočne hrane

Nakon uspjelog međunarodnog savjetovanja KRMIVA '93 (29 referata od čega 11 inozemnih, preko 140 sudionika industrije krmnih smjesa i ostalih zainteresiranih), u pripremi je međunarodno savjetovanje KRMIVA '94.

Za međunarodno savjetovanje KRMIVA '94 navodimo osnovne podatke:
- dani održavanja
  8. lipnja (srijeda)
  9. lipnja (četvrtak)
- mjesto održavanja: Opatija
- tema na savjetovanju: tehnologija proizvodnje krmnih smjesa
  hranidba svinja, peradi, riba i sitnih preživača
  priprema hrane na gospodarstvima i njegina primjena.

After successful international conference KRMIVA '93 (29 topics, more than 140 participants from feed milling industry and others), the Association is now preparing the next Conference - KRMIVA '94.

For the Conference KRMIVA '94 we can give basic data as follows:
- time: June 8 - 10, 1994.
- venue: Opatija - famous seaside resort on Adriatic coast
- theme: feed milling technology,
  animal nutrition (swine, poultry, fish, small ruminants)
  preparing the feed on farms and its application.

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