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IMMUNE MODULATION OF CHICKENS BY DIETARY CALCIUM AND LEAD

IMUNA MODULACIJA PILIĆA DIJETOM KALCIJA I OLOVA

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Izvorni znanstveni članak
UDK: 636.5:636.085.12
Primljeno: 1. prosinca 1993.

Summary

Male chicks (Peterson x Arbor Acres) were obtained from a commercial hatchery and fed diets containing 0, 1, 10 and 100 ppm lead at two levels of calcium (0.65 and 1.30%). The chickens were maintained free of adventitial infections and vaccinated for infectious bursal disease at 10 and 18 days of age, and for Newcastle disease at 21 days of age. Antibody titers for Newcastle disease, measured by enzyme-linked immunosorbent assay, were significantly higher at six weeks in groups fed high level of calcium. Low levels of lead significantly increased and the highest level (100 ppm) suppressed titers. The stimulatory effects of calcium and lead were additive. Titers for infectious bursal disease virus were not significantly altered. Relative bursal weights were increased and decreased in concordance with antibody titers for Newcastle disease virus. Morphometric analysis of bursal tissue sections revealed that the areas of bursal follicles, their cortices and medullae were increased in groups that had increased relative bursal weights.

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and Bosna-Herzegovina for the decade preceding the current war revealed significant immune suppression (Ragland et al., unpublished observations).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

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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 withrawal period prior to slaughter.

Studies conducted by Bakalli and colleagues in Prishtina to evaluate toxic effects of heavy melats 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 adventitial 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 $PbSO_4$) 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-BLEN™, CEVA Laboratories, Overland Park, Kansas) and at 21 days of age for Newcastle diesease (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 BU-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 weigths 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 inmedullary 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 diferentiation, 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 constistent with enhanced immune responsiveness.

These effects on immune responsiveness may be mediated through an effect on 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 adenylated cyclase transduction systems (Lawrance, 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 recomended. 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).
Tablica 1 - Utjecaj olova i kalcija u hranidbi na titre protutijela za zaraznu bursalnu bolest i Newcastle bolest kod brojlera (pokus 1)

Dietary lead (ppm) Olovo u hrani		Dietary Calcium (%) Kalcij u hrani		
		0.65	1.3,	Mean[1] ± S.E. Prosjek ± S.E.
		Mean (n) Prosjek (n)	Mean (n) Prosjek (n)	
0	IBD	5495 (30)	1323 (23)	3685[ab] ± 605
	NCD	8604 (30)	16350 (23)	11965 ± 2861
1	IBD	8441 (29)	2224 (23)	5691[a] ± 1222
	NCD	11866 (29)	26940 (23)	14110 + 2784
10	IBD	3491 (29)	2365 (22)	3005[b] + 529
	NCD	12083 (29)	19321 (22)	15205 + 3322
100	IBD	3535 (30)	2630 (22)	3152[b] ± 647
	NCD	10219 (30)	28060 (22)	17767 + 4893
Mean ± SE[1] Prosjek ± SE[1]	IBD	5228[a] ± 639	2128[b] ± 335	
	NCD	10671[a] ± 1523	20089[b] ± 3518	

[1] Main effect means with different superscripts are significantly different, $p \leq 0.05$

Table 2 - Influence of dietary lead and calcium on the body weight and relative bursa weight of 42-day old broiler chickens (experiment 1)
Tablica 2 - Utjecaj olova i kalcija u hranidbi na tjelesnu težinu i relativnu težinu burze brojlera starih 42 dana (pokus 1)

Dietary lead (ppm) Olovo u hrani		Dietary Calcium (%) Kalcij u hrani (%)		
		0.65	1.3,	Mean[1] ± S.E. Prosjek[1] ± S.E.
		Mean (n) Prosjek (n)	Mean (n) Prosjek (n)	
0	Weight gain (kg) Težinski prirast (kg)	2.067 (3)	1.977 (3)	1.992± .014[a]
	Bursa weight (%) Težina burze (%)	.188 (10)	.177 (10)	.152 ± .014[b]
1	Weight gain (kg) Težinski prirast (kg)	1.920 (3)	1.990 (3)	1.955± .021[ab]
	Bursa weight (%) Težina burze (%)	.237 (10)	.209 (10)	.224 ± .015[a]
10	Weight gain (kg) Težinski prirast (kg)	1.920 (3)	1.893 (3)	1.907± .016[bc]
	Bursa weight (%) Težina burze (%)	.229 (10)	.257 (10)	.243 ± .02[a]
100	Weight gain (kg) Težinski prirast (kg)	1.893 (3)	1.857 (3)	1.875 ± .014[a]
	Bursa weight (%) Težina burze (%)	.228 (10)	.260 (10)	.224 ± .02[a]
Mean ± SE[1] Prosjek ± SE[1]	Weight gain (kg) Težinski prirast (kg)	1.935±0.016	1.929 +019	
	Bursa weight (%) Težina burze (%)	.220 ±0.011	.210+016	

[1] Main effect means with different superscripts are significantly different, $p \leq 0.05$

Table 3 - Morphometrics analysis of bursa of Fabricius from broiler chickens fed lead and calcium (mean areas in thousands of micrometers).(Experiment 2)
Tablica 3 - Morfometrička analiza burze Fabricius brojlera hranjenih olovom i kalcijem (prosječna područja u tisućinkama mikrometara) (pokus 2)

ppm Pb	% Ca	Follicle	Medulla (M)	Cortex (C)	C/M
0	0.65	38	19	19	1.01
	1.30	46[a]	28[a]	18	0.65[a]
1	0.65	39	20	19	0.96
	1.30	44[a]	26[a]	18	0.68[a]
10	0.65	47	27	20	0.78
	1.30	59[a]	35[a]	24	0.69[a]
100	0.65	41	22	19	0.86
	1.30	68[a]	40[a]	28[a]	0.71[a]

[a] Means are significantly different from 0.65% calcium at level of $p < 0.05$.

Table 4 - Influence of dietary lead and calcium on antibody titers for Newcastle disease (experiment 2)
Tablica 4 - Utjecaj olova i kalcija u hranidbi na titre antitijela za Newcastle bolest (pokus 2)

Dietary lead (ppm) Olovo u hrani	Dietary calcium (%) Kalcij u hrani (%)		Mean ±S.E. Prosjek ± S.E.
	0.65	1.30	
0	6424 ± 1334	15833 ± 5188	11128 ± 3188
1	9470 ± 4379	16379 ± 1333	12924 ± 2565
10	12744± 2878	17835 ± 4198	15290 ± 2545
100	14422 ± 7158	16438 ± 9380	15431 ± 5296
Mean[1] ± SE Prosjek[1] ± SE	10765[a] ± 2125	16621[b] ± 2481	

[1] Main effect means with different superscripts are significantly different, $p \leq 0.08$

Table 5 - Influence of dietary lead and calcium on antibody titers for Infectious bursal disease (experiment 2)
Tablica 5 - Utjecaj olova i kalcija u hranidbi na titre antitijela za zaraznu burzalnu bolest (pokus 2)

Dietary lead (ppm) Olovo u hrani	Dietary calcium (%) Kalcij u hrani (%)		Mean ±S.E. Prosjek ± S.E.
	0.65	1.30	
0	8859 ± 1618	7664 ± 261	8262 ± 780
1	14587± 2893	9109 ± 1294	11848 ± 2565
10	8909 ± 293	12998 ± 2154	10953 ± 1334
100	9385 ± 1832	13054± 2638	11215 ± 1653
Mean ± SE Prosjek ± SE	10435 ± 1087	10704 ± 1058	

CONCLUSIONS

Antibody response to Newcastle disease vaccine but not infectious bursal disease vaccine is greater in chickens fed 1.3 than0,65% 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 Europen Assistance Awards Program administered by NAFSA:Association of International Educators.

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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 antitijela za Newcastle bolest mjereni imunosorbentnim uzorkom vezanim enzimom bio je znatno veći (viši u šestom tjednu u skupinama hranjenim visokim razinama kalija. Niske razine olova (0; 1 i 10 ppm) znatno su porasle a najviše (100 ppm) su razine potisnule titer. Dodatni su bili stimulativni učinci kalcija i olova. Titri za virus zarazne bursalne bolesti nisu se značajno izmijenili. Relativne bursalne težine povećavale su se i smanjivale u skladu s titrima antitijela 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.

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