

EFFECT OF DIETARY LUTEIN ON IMMUNE RESPONSE OF BREEDERS AND BROILER CHICKS

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INTRODUCTION

Lutein is a xanthophyll carotenoid found naturally in marigold flowers, corn, and dark-green leafy vegetables. Recent evidence suggests that lutein may be beneficial in the protection against numerous human diseases, such as macular degeneration, heart disease, and cancer (Ribaya-Mercado and Blumberg, 2004). Due to its suggested role in disease prevention, research has been conducted on the development of lutein-enriched eggs, through supplementation of the layers' diet (Leeson and Caston, 2004). Since lutein is a fat-soluble compound, the lutein composition of eggs from layers can be manipulated by adjusting the level of lutein in the layers' diet (Leeson and Caston, 2004).

In terms of the effect of lutein on the immune system, studies conducted on cats (Kim et al., 2000) and dogs (Kim et al., 2000) have found dietary lutein to have a stimulatory effect on both the cell-mediated and humoral immune responses. Similarly in mice, lutein was found to stimulate antibody production in response to an antigen (Jyonouchi et al., 1994). As observed in these other species, Bédécarrats and Leeson (paper submitted to the Journal of Applied Poultry Research) have shown that dietary lutein can also enhance the immune response in layers.

The objectives of this study were to determine the effects of dietary lutein on the immune response of broiler breeders following vaccination. As well, to determine whether lutein in the breeders' diet would have an effect on the passive transfer of maternal antibodies, and hatchability of chicks.

MATERIALS AND METHODS

Animals and Diets

Birds used for this study were Ross Ross broiler breeders. Birds were raised in collective floor pens at Arkell Poultry Farm (Arkell, ON). At 35 weeks of age, birds were randomly assigned to 6 experimental groups of 20 female and 2 male breeders (n=22 per group) per pen. Starting at 36 weeks of age, birds were fed a standard breeders' diet supplemented with 0, 30, 60, 90, 120, or 150 ppm lutein (Table 1). The experimental diets were fed for a period of 30 days before the first blood collection and vaccination took place, and until the end of the trial thereafter (Figure 1).

Antibody Response of Broiler Breeders

Breeders were vaccinated against Infectious Bronchitis virus (IBV), Newcastle, and Gumboro (an infectious bursal disease) at 40 weeks of age via an intramuscular injection (Breedervac- IV- Plus). Blood samples were collected from all breeders one day prior to and 15 and 29 days following vaccination. Plasma was separated by centrifugation and frozen at -80EC until antibody titre assays could be performed. To evaluate immune response to vaccination, antibody titres for IBV, Newcastle, and Gumboro were measured by ELISA at the Animal Health Laboratory (University of Guelph, Guelph, ON).

Egg Incubation and Analysis

Eggs were collected for one week starting 1 day after the final blood collection (29 days post vaccination). At the time of egg collection, hens were 44 weeks of age and had been fed the experimental diets for a period of 61 days. In order to measure antibody transfer from hen to egg, 10 eggs per treatment were cracked (n=60), yolks were separated from the albumen, diluted, and antibody titres for IBV, Newcastle, and Gumboro were measured by ELISA.

To measure lutein transfer efficiency from the diet to the egg, yolk colour was assessed in 5 eggs per treatment (n=30) using the Roche colour scale of 1 to 15. In addition, lutein content in the yolk was measured by high performance liquid chromatography (HPLC).

The remaining eggs were set for incubation in an artificial incubator. The number of eggs set, and number of chicks hatched for each treatment was recorded.

Antibody Response and Organ Collection from Chicks

Prior to vaccination at one day of age, 10 chicks per treatment (n=60) were randomly selected, weighed, and then euthanized by CO₂ gas for blood and organ collection. All organs (bursa, spleen, thymus, and liver) were weighed and corrected for body weight, while blood was kept for antibody titre analysis. The remaining chicks were sprayed with live IBV vaccine (Merial Select) and injected with Marek's disease live virus vaccine (MD-Vac CFL, Fort Dodge). Chicks were housed in floor pens according to the diet the breeders received. At 15 days of age, 10 chicks were randomly chosen from each treatment (n=60) for blood collection. Plasma from all samples was separated by centrifugation and frozen at -80EC until antibody titre assays could be performed for IBV, Newcastle, and Gumboro by ELISA.

RESULTS AND DISCUSSION

Antibody Response of Broiler Breeders

Prior to vaccination (day 0), baseline IBV antibody titres were higher in birds fed 120 and 150 ppm lutein (treatments 5 and 6), than birds fed lower levels of lutein (Table 2). At 15 days post vaccination, birds in treatments 1 through 4 responded to vaccination with an increase in antibody titre. However, birds in treatments 5 and 6 did not respond to vaccination, indicated by a slight decrease in titre. These results were quite surprising, and contradict what was previously observed in layers by Bédécarrats and Leeson (submitted to the Journal of Applied Poultry Research). Although not included in the original protocol, it later appeared that all birds were vaccinated against IBV nine days before the start of the trial as part of Arkell farm's standard operating procedure when birds are moved from one barn to another. This unanticipated added vaccination can explain the abnormally high antibody levels observed in all groups at day 0. Interestingly, titres appeared significantly higher in the groups fed higher levels of lutein. It is thus possible that lutein fed during the first 30 days helped maintain higher antibody levels for a longer period of time. Furthermore, these initial higher titres may have neutralized the vaccination at 40 weeks, thus inhibiting the increase in antibody titre in these 2 groups.

Antibody responses of groups to Gumboro vaccine are shown in Table 3. Titres for all experimental groups were low prior to vaccination (day 0), indicating that birds had not been previously vaccinated or exposed to the virus. Birds in all groups responded to vaccination, seen as an increase in antibody titre at 15 days post vaccination. At 29 days post vaccination, antibody titres for treatments 1 through 4 decreased, while treatments 5 and 6 experienced an increase. These results suggest that higher levels of lutein may help to maintain antibody levels at an elevated level for a longer period of time. This is similar to results found by Bédécarrats and Leeson (paper submitted to the Journal of Applied Poultry Research), who found that layers fed a diet containing 125 ppm lutein had a sustained secondary immune response. Unfortunately, blood samples were not collected past 29 days post vaccination. Therefore, it is unknown whether the antibody titres of groups fed higher levels of lutein would have remained elevated.

Antibody responses of groups to Newcastle vaccine are shown in Table 4. All treatments had low antibody titres prior to vaccination, and an increase in titre was seen for all treatments 15 days post vaccination. At 29 days post vaccination, treatments 4 and 6 experienced a smaller decline in antibody titre than treatments 1, 2 and 3, while birds in treatment 5 continued to experience an increase in antibody titre. As seen with the response to Gumboro vaccination, the higher levels of dietary lutein may help maintain antibody titres for a longer period of time.

Egg Yolk Analyses

The lutein content in eggs from broiler breeders was measured to ensure that lutein was being transferred from hen to egg. As shown in Table 5, lutein content in the egg yolk did increase as the level of lutein in the diet increased (Table 5). However, the highest level of lutein was found in eggs from treatment 5 (120 ppm lutein), not from treatment 6 (150 ppm lutein). Leeson and Caston (2004) also observed that at higher levels of inclusion in the diet, transfer efficiency of lutein from hen to egg decreases, with maximum transfer efficiency around 125 ppm lutein.

Results for the assessment of yolk colour using the Roche colour scale are shown in Table 5. The inclusion of lutein in the diets is indicated by a higher number (darker colour) on the Roche colour scale, in comparison to the control group. Since lutein is a carotenoid pigment, it is incorporated into the egg yolk, causing a change in colour (Leeson and Caston, 2004).

Antibody levels of egg yolks were measured in order to determine the effect of lutein in the hens' diet on the passive transfer of maternal antibodies from hen to egg. As shown in Table 6, no significant differences were observed. These results are not surprising, since no difference in titres were observed between hens at the time of egg collection. However, as mentioned in the previous section, the data was collected prematurely and differences might have occurred at a later stage.

Hatchability

Hatchability of eggs from birds fed lutein was higher than the control group (Table 7). Treatments 2 through 5 had hatchability values greater than 90%. These values are higher than the expected hatchability of 80% for broiler breeders at 44 weeks of age. The abnormally low hatchability (65%) observed in the control group could be explained by a lower fertility rate, since only 2 males were present per pen, and one of the males in this group had to be housed outside due to aggressive behaviour. However, the high level of hatchability in the lutein groups is very encouraging, although, since eggs were not candled, it is unknown whether it is the result of higher fertility or lower embryonic mortality.

Organ Weights of Chicks

Organs were collected and weighed to determine if lutein in the breeders' diet would have an effect on the organ size of the chicks, especially lymphoid organs. A ratio of organ weight to body weight was calculated in order to account for differences between chicks in size/weight (Table 8). No significant differences were found between treatments ($p>0.05$).

Antibody Response of Chicks

As shown in Table 6, no significant differences were observed between treatments. However, these results are not surprising since there were no differences in antibody titres between hens and egg yolks at the time of egg collection.

CONCLUSION

The health of broiler breeders and their offspring is an important issue in poultry production, which is why it is necessary to investigate ways to improve the health status of these birds. Based on results from this study, lutein may prove to be an effective way to improve the immune status of broilers, by maintaining antibody levels for a longer period of time after vaccination. A prolonged immune response may benefit the broiler industry by increasing the interval between vaccinations, or by reducing the number of vaccinations required. Other results from this study indicate that lutein may improve hatchability, another potential benefit to the broiler industry. In conclusion, since this is a new area of research, further studies should be conducted to better understand the effect of dietary lutein on the immune response of broiler breeders and their offspring, as well as on the hatchability of eggs.

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REFERENCES

Bédécarrats, G.Y., and S. Leeson. Dietary lutein influences immune response in laying hens. Paper submitted to the Journal of Applied Poultry Research.

Jyonouchi, H., Zhang, L., Gross, M., and Y. Tomita. 1994. Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. *Nutr. Cancer*. 21:47-58.

Kim, H.W., Chew, B.P., Wong, T.S., Park, J.S., Weng, B.B.C., Byrne, K.M., Hayek, M.G., and G.A. Reinhart. 2000. Dietary lutein stimulates immune response in the canine. *Vet. Immunol. Immunopathol.* 74:315-327.

Kim, H.W., Chew, B.P., Wong, T.S., Park, J.S., Weng, B.B.C., Byrne, K.M., Hayek, M.G., and G.A. Reinhart. 2000. Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. 73:331-341.

Leeson, S., and L. Caston. 2004. Enrichment of eggs with lutein. *Poult. Sci.* 83:1709-1712.

Ribaya-Mercado, J.D., and J.B Blumberg. 2004. Lutein and zeaxanthin and their potential roles in disease prevention. *J. Am. Coll. Nutr.* 23:567S-587S.

Figure 1. Outline of Project

Effect of Dietary Lutein on Immune Response of
 Breeders and Broiler Chicks
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<u>Broiler Breeders</u>	<u>Age of Birds</u>	
Day 1	36 weeks	Experimental diets introduced
Day 31	40 weeks	Blood samples collected from all birds, followed by vaccination against IBV, Newcastle, and Gumboro
Day 46	42 weeks	Blood samples collected from all birds
Day 60	44 weeks	Blood samples collected from all birds
Day 61-67	44-45 weeks	Eggs collected for incubation and assessment of yolk colour, lutein content, and antibody titres
<u>Broiler Offspring</u>		
Day 1	1 day	Blood samples and organs (bursa, spleen, thymus, liver) collected from 10 chicks/ treatment
Day 15	15 days	Blood samples collected from 10 chicks/treatment

Table 1. Diet Composition

Treatment	Dietary Lutein (ppm)
1	0
2	30
3	60
4	90
5	120
6	150

Table 2. Effect of dietary lutein on IBV antibody titres of broiler breeders. Results are expressed as mean antibody titre " SEM.

Treatment	Days after IBV vaccination		
	0	15	29
1	9998 " 911	14320 " 1229	10876 " 687
2	9787 " 1037	13721 " 1255	11304 " 920
3	4455 " 657	7534 " 647	6347 " 566
4	7776 " 772	11776 " 932	11086 " 746
5	16202 " 1132	9552 " 949	9195 " 784
6	13427 " 1284	9870 " 699	9826 " 559

Table 3. Effect of dietary lutein on Gumboro (infectious bursal disease) antibody titres of broiler breeders. Results are expressed as mean antibody titre " SEM.

Treatment	Days after Gumboro vaccination		
	0	15	29
1	148 " 86	4695 " 641	4296 " 502
2	1 " 0	6594 " 904	5432 " 848
3	58 " 45	5098 " 628	4818 " 461
4	72 " 63	4914 " 758	3908 " 424
5	154 " 53	5824 " 823	6213 " 746
6	80 " 19	4280 " 753	4912 " 898

Table 4. Effect of dietary lutein on Newcastle antibody titres of broiler breeders. Results are expressed as mean antibody titre " SEM.

Treatment	Days after Newcastle vaccination		
	0	15	29
1	965 " 179	16266 " 464	14359 " 388
2	831 " 101	16420 " 621	14277 " 471
3	1124 " 166	15262 " 609	13949 " 417
4	1533 " 284	15074 " 484	14673 " 255
5	1444 " 273	13839 " 875	14431 " 339
6	954 " 158	12881 " 1246	11907 " 1090

Table 5. Lutein content and Roche colour score of egg yolks.

Treatment	Lutein Content (ug/g yolk)	Roche Colour Score
1	2.015	6.6
2	17.233	9.4
3	32.673	10.4
4	38.378	11
5	58.326	11.7
6	50.075	11.6

Table 6. Antibody titres of egg yolks and day old chicks. Results are expressed as mean

antibody titre " SEM.

Treatment	Yolk			Chick		
	IBV	Gumboro	Newcastle	IBV	Gumboro	Newcastle
1	12030 " 816	4751 " 539	14679 " 329	7735 " 960	1744 " 245	13484 " 297
2	13225 " 1497	5489 " 824	15023 " 344	8102 " 1476	3788 " 637	13300 " 781
3	8219 " 786	7574 " 737	15700 " 482	3440 " 549	2808 " 442	13888 " 231
4	14382 " 722	7266 " 1099	16256 " 300	10665 " 1294	2683 " 547	13713 " 462
5	12617 " 982	6743 " 624	15309 " 411	7910 " 1001	3473 " 277	12950 " 417
6	13104 " 990	5256 " 1451	11714 " 2107	6301 " 1157	1978 " 877	10400 " 1746

Table 7. Effect of lutein in breeders' diet on hatchability.

Treatment	Hatchability ^A (%)
1	64.6
2	93.7
3	94.0
4	91.7
5	91.4
6	77.2

^AHatchability = (number of hatched eggs / number of eggs set) x 100

Table 8. Effect of lutein in breeders' diet on organ size in day old chicks. Values were

calculated as a ratio of organ weight to chick body weight.

Treatment	Body weight (g)	Organ weight : Body weight ratios			
		Bursa	Spleen	Thymus	Liver
1	41.9	0.00115	0.000437	0.00111	0.0284
2	40.2	0.00105	0.000552	0.00154	0.0285
3	41.4	0.00128	0.000575	0.00132	0.0273
4	40.3	0.00120	0.000577	0.00130	0.0263
5	42.6	0.00126	0.000420	0.00158	0.0274
6	40.1	0.00119	0.000459	0.00156	0.0312