Factors Affecting The Incidence of Blood Spots In Eggs

ELBERT J. DAY, BEN C. DILWORTH and O. W. CHARLES

Mississippi State University AGRICULTURAL EXPERIMENT STATION

HENRY H. LEVECK, Director

STATE COLLEGE

MISSISSIPPI

Summary and Conclusion

The incidence of egg blood spots in eggs was altered (deceased and increased) by modifying the diet of cage layers.

Addition of alfalfa meal (a rich source of vitamin K) or MSBC (synthetic vitamin K) to the diet tended to increase the number of eggs containing blood; while, addition of anti-vitamin K compounds decreased the incidence of egg blood spots.

Within a short range in blood prothrombin time, there appears to be an inverse relationship between egg blood spotting and prothrombin time. More research work is needed to determine the exact cause of egg blood spots and to find ways to prevent their occurrence.

FACTORS AFFECTING THE INCIDENCE OF BLOOD SPOTS IN EGGS

ELBERT J. DAY, BEN C. DILWORTH and O. W. CHARLES

Blood spots in eggs result in a great economic loss to the poultry industry. It has been estimated that from 1 to 3% of all commercial eggs are discarded because they contain blood spots. An additional 1-3% of the eggs passing the candling inspection contain very small, pin-point spots too small to be detected by the candling procedure. Such eggs undoubtedly reduce per capita consumption because of the unfavorable impression they create.

Blood spots are thought to be caused by hemorrhages in the follicles of the hen's ovary. These ruptures are thought to occur prior to ovulation, during ovulation and following ovulation. The basic cause of the hemorrhages is not known. It would be logical to assume that factors which affect capillary fragility (vitamin C) or blood clotting (vitamin K) would also influence the incidence of blood spots in eggs.

Early attempts to correct this abnormality centered around these suppositions. Birds were fed fresh young grasses, allowed to range or fed dehydrated alfalfa meal in attempts to reduce the number of eggs containing blood. Due to the favorable reports from feeding grasses and alfalfa meal, vitamins K, E, D, A and C were tested as well as added phosphate and rutin, but all were ineffective.

Recent trials at several experiment stations with added alfalfa meal, a good source of Vitamin K, have also been ineffective in reducing egg blood spots. It is becoming increasingly apparent that vitamin K deficiencies are not responsible for the spots. The addition of synthetic vitamin K active compounds or the addition of feedstuffs high in vitamin K, such as alfalfa meal, usually exert no favorable effect.

Although efforts to reduce the incidence of spots by nutritional feed additives have in general failed, it has been well established that adequate nutritional balance, is important. A dietary deficiency of vitamin A or excess protein has been shown to aggravate egg blood spotting. Management practices or physiological stress is another important factor. For instance, birds housed in raised wire cages lay eggs containing approximately 33% more blood spots than comparable birds maintained on litter floors. Genetic selection has been practiced extensively as a method of spot incidence, but it has not been completely successful in eliminating them in eggs.

The trend toward increased numbers of cage layers and the advent of some recent interesting research results have served to increase the research efforts directed toward finding ways to reduce the incidence of blood spots in eggs.

Recent research results which have been of particular interest can be summarized rather briefly: (1) Alfalfa meal, which is known to be the best natural source of vitamin K, has been found by two different research groups (Mississippi and Idaho) to increase blood spots in eggs when added to layer diets and (2) vitamin K antagonists, such as dicumarol, have been found to decrease the incidence of egg blood spotting.

These findings are certainly contrary to what one might logically conclude from our past beliefs about the primary cause of egg blood spots. Since vitamin K is necessary for the biosynthesis of prothrombin, an enzyme essential in the blood clotting process, it was generally

^{*}Staff members, Poultry Science Department, Mississippi Experiment Station.

thought that abundant dietary levels of viamin K were beneficial, not harmful, to the egg blood spotting problem. Apparently, there is no correlation between the bird's prothrombin time and blood spotting of eggs.

In studies conducted by research workers at the University of Florida and at our station, prolonged prothrombin times, which indicate a slow blood clotting time, have not resulted in an increased number of eggs which blood spots as one might suspect. At our station, vitamin K antagonists have been found to actually reduce the number of eggs containing blood spots, while vitamin K active compounds and alfalfa meal have been found to aggravate this condition. The purpose of this paper is to present some of our findings in this area.

Experimental Procedure

White Leghorn layers were maintained in individual cages and allowed feed and water on a free choice basis. The dietary additives were substituted into corn-soy type basal diets (Table 1). The experimental variables are shown in Tables 2, 3, 4 and 5 along with the results from each study. The incidence and size of blood spots were determined by collecting and breaking all eggs laid during two consecutive days each week. A glass plate mirror device was used which allowed us to view the broken - out eggs

from all angles. Blood prothrombin determinations were made at the end of each test period. Replicate or quadruplicate groups of 17 birds per group were used to evaluate each dietary treatment in all tests.

Results and Discussion

Since egg production and feed utilization were not significantly affected by dietary treatment, these data are not presented for Trial 1. Blood spotting of eggs and blood prothrombin times were affected by the types of diets fed, as shown in Table 2. The single dietary addition of the active vitamin K supplement, menadione sodium bisulfite compler (MSBC), significantly increased the percentage of eggs containing blood spots as compared to the control diet. Alfalfa meal also tended to cause a higher incidence of blood spots, but this increase was not significantly different from the

Table 1. Composition of basal diets.

	Percentage			
Ingredient	Exp. 1	Exp. 2-5		
Yellow corn	72.34	70.00		
Soy, 50% protein	20.00	21.47		
Limestone	4.50	5.00		
Defl. phos.		Section 2		
14% P. 29% Ca	2.50	2.40		
Sodium chloride	0.40	0.40		
Premix	0.23	0.631		
Poultry fat		0.50		
TOTAL	100.00	100.00		

¹Contributes 0.025% DL-methionine in addition to vitamins and trace minerals.

Table 2. Effect of supplemental menadione sod um bisulfite complex, (MSBC) alfalfa meal and dried whey on the incidence of blood spots in ergs and blood prothrombin time of layers, Experiment 1.

Diet	Number of eggs broken	Number of blood spots	Blood spots, %	Blood prothrombin time, sec.
Basal	1985	80	4.0 a ¹	46.0 a ¹
+ MSBC, 4 g./ton	1968	136	6.9 b	34.2 bc
+ 17% alfalfa, 2.5%	1874	96	5.1 ab	36.9 bc
+ 17% alf., $2.5% + MSBC$	1819	96	5.3 ab	34.6 bc
+ whey, F.M. and alfalfa	1882	98	5.2 ab	41.4 ab
+ whey, F. M., alf. & MSBC	1983	107	5.4 ab	33.4 c

¹Averages within a column and without a common letter designation are significantly different at the 5% level of probability.

average percentage blood spots of eggs from hens fed the basal diet.

The average blood prothrombin time for the birds fed the simplified corn-soy basal ration was significantly lower than that of the other groups. These data suggest that a rise in blood Prothrombin time may be beneficial, rather than harmful, in preventing blood spotting of eggs.

The second test (Table 3) was designed primarily to determine whether or not there was a correlation between egg blood spotting and blood prothrombin of layers. Dicumarol was added to the diet to prolong prothrombin time and MSBC was added to reduce the prothrombin time.

Dicumarol significantly reduced the Percentage of eggs containing blood spots. In addition, blood prothrombin time was significantly increased by feed

ing dicumarol. The single dietary additions of MSBC or sulfaquinoxaline (SQ) had no significant effect on blood prothrombin time or egg blood spotting. Both additives tended to increase the number of eggs with blood spots.

The addition of both MSBC and SQ to the basal caused a significant change in egg blood spot incidence without significantly affecting prothrombin time. SQ was the only additive which significantly reduced egg production and/or feed utilization. As would be expected, the addition of MSBC to the diet decreased the adverse effect of SQ on performance.

Experiment 3 was conducted primarily to determine the relative potency of several available vitamin K antagonists. We believed that it was imperative for us to find a compound more potent and cheaper than dicumarol. Such a compound was available as can be seen from the

Table 3. The effect of menadione sedium bisulfite complex (MSBC) dicumarol and sulfaquinoxaline (SQ) on the incidence of egg blood soots and blood prothrombin time of cage layers, Experiment 2.

_		:		
Dietary treatment ²	Egg blood spots, %	Prothrombin time, sec.	Hen-day	Lbs. feed doz. eggs
Basal plus MSBC Basal plus dicumarol Basal plus SO Basal plus SQ and MSBC	4.78 a 5.89 a 1.58 b 5.37 a 9.02 c	29.2 ab 28.9 ab 53.5 c 35,1 b 27.9 a	68.8 a 58.2 a 66.2 a 50.8 b 52.4 b	4.59 a 4.56 a 4.73 ab 5.27 b 5.12 ab

²Additive levels were: dicumarol, 100 mg./lb.; SQ, 56.5 mg./lb., and MSBC, 2 mg./lb. (SQ was fed at 113.0 mg./lb. during the first month.

Table 4. A comparison of the anti-vitamin K activity of different compounds, Experiment 3.

Dietary modification		Two month resu'ts ¹			
Additive	Mg./lb.	Prothrombin time, sec.	Total eggs	Blood spots %	
Basal + Dicumarol + Dicumarol + Dicumarol + Dicumarol + Dicumarol + Dicumarol + Diphacinone + Pivalyl + Fumarin-22 ² ¹ See footnote 1, Table 1.	100.00 50.00 12.50 3.12 1.56 12.50 12.50	27.3 a 38.3 a 39.5 a 31.8 a 29.7 a 31.1 a 64.6 b 84.1 b 31.2 a	313 360 357 305 318 364 294 326 393	4,90 a 1.58 a 2.00 a 1.55 a 6.24 a 2.48 a 3.56 a 2.12 a 2.42 a	

²The basal diet was substituted for the Fumarin-22 ration five days prior to the blood sampling,

data presented in Table 4. Pivalyl and diphacinone, two indandione derivations, are much more potent than dicumarol. Critical chick bioassays at our laboratory have shown that pivalyl and diphacinone are 20 and 15 times, respectively, more potent vitamin K antagonists than dicumarol. Since pivalyl costs less and is more potent than the other compounds it was used along with dicumarol in subsequent layer tests.

Results from the fourth experiment are quite typical of most of the previous data obtained at our station (Table 5). Both alfalfa meal and MSBC tended to increase the incidence of blood spots in eggs. The percentage blood spots in eggs from hens fed pivalyl (5 mg./lb.) was significantly reduced as compared to eggs from hens fed MSBC (2 mg/lb.). The percentage blood spots also tended to be less from hens fed either pivalvl at the higher dietary level (10 mg/lb.) or dicumarol (100 mg./lb.). Feeding the highest dietary level of pivalyl (10 mg/ lb.) resulted in an extremely long prothrombin time, but was not more beneficial in reducing egg blood spots than the lower dietary level (5 mg/lb.).

In the final experiment to be reported in this paper, an attempt was made to further increase the incidence of egg

blood spotting by adding a very high dietary level of MSBC (20 mg./lb.) and also determine whether or not a very low dietary level of pivalyl (0.5 mg/lb.) would reduce egg blood spotting.

Results from the last experiment are presented in Table 6. These data are somewhat different from the previous results because supplemental alfalfa meal (5%) and MSBC did not aggravate the egg blood spot problem. The data from this experiment are similar in one respect to the previous results since the highest level of pivalyl again significantly reduced the percentage of eggs containing blood. The failure of added alfalfa meal and MSBC to aggravate the egg blood spot problem in this test could be related to the blood prothrombin time of the birds. Usually the birds fed the corn-soy basal ration have exhibited an

Table 5. The effect of diet on prothrombin time and incidence of blood spets, Experiment 4.

with including of proof	speis, Experiment To			
Dietary modifications	Prothrombin time, sec. 1	% blood spots1		
Basal ,	33.1 a	4.2 ab		
+ Alfalfa. 2.5%	30.0 a	5.1 ab		
+ MSBC, 2 mg./lb. ,	, 29.4 a	6.6 a		
+ Dicumarol, 100 mg./lb	66,8 Ь	2.1 ab		
+ Pivalyl, 5 mg./lb.	63.6 b	1.6 b		
+ Pivalyl. 10 mg./lb.	133.4c	2.3 ab		
1Sec footnote 1. Table	1			

Table 6. Effect of dietary modifications on performance, prothrombin time and egg blood spotting.

Experiment 5.

		-inportane it 5.			
. D'		Ro	sults, 9 mon	ths ¹	
Dietary modifications	% Lay	Lbs. feed/ doz. eggs	% Mort.	Prothrombin time, sec.	% blood spots
Basal + 17% Alfalfa (5%)	63.5 a ¹ 56.1 b	4.6 b ¹ 5.0 a	9.6 8.0	20.8 a ¹ 20.4 a	4.8 a ¹ 4.5 a
+ MSBC (2 mg./lb.)	65.7 a	4.4 bc	8.0	19.5 a	4.0 a
+ MSBC (20 mg./lb.)	63.4 a	4.5 Ъ	14.4	18.6 a	4.0 a
+ Pivalyl (.5 mg./lb.)	66.4 a	4.2 c	6.4	22.1 a	5.1 a
+ Pivalyl (5 mg./lb.)	66.6 a	4.5 Ъ	17.6	31.1 ь	1.3 Ь

¹See footnote 1, Table 1.

average blood prothrombin time of 27-33 seconds, but in the last test, prothrombin time for the control birds was 20.8 seconds. We have no explanation for this difference in prothrombin time of the control birds at present. The experimental procedure of determining blood prothrombin time was held constant for all tests, but it is highly likely that there is some variation in the vitamin K content of the ingredients used in the basal ration from experiment to experiment. Also, differences in intestinal synthesis of vitamin K could account for variation in the prothrombin time of the birds fed the basal diet.

These data suggest that if there is an inverse relationship between egg blood spotting and blood prothrombin time, this relationship extends only for a short range in prothrombin time. Severe pro-

longation of the prothrombin time was no more beneficial in reducing egg blood spotting than a more moderate level. Also, high dietary levels of added MSBC do not appear to be any more detrimental than low levels of added MSBC.

Except during the first experiment, all egg blood spots were recorded according to size and a percentage distribution table was made for each experiment as is indicated in Table 7 for the last experiment. It is apparent from these data, which are representative of the results from the other experiments, that the additive did not materially affect the size distribution pattern of the blood spots. We had assumed that the anti-vitamin K compounds might be effectively reducing the number of egg blood spots detected by hastening disolution, especially the pin-point size blood spots.

Table 7. The effect of diet on the size of egg blood spots, Experiment 5.

Egg blood spots, %			
Pin-point	-1/8"	+1/8"	Bloody
32.8	25.0	39.0	3.2
41.2	21.8	35.4	1.6
22.6	34.7	42.7	0.0
34.6	26.6	37.8	1.0
35.9	19.6	41.5	3.0
38.3	21.7	40.0	0.0
	32.8 41.2 22.6 34.6 35.9	Pin-point —1/8" 32.8 25.0 41.2 21.8 22.6 34.7 34.6 26.6 35.9 19.6	Pin-point 1/8" +-1/8" 32.8 25.0 39.0 41.2 21.8 35.4 22.6 34.7 42.7 34.6 26.6 37.8 35.9 19.6 41.5