PROBLEMS ASSOCIATED WITH THE FEEDING OF RATIONS CONTAINING RAPESEED MEAL TO LAYING CHICKENS

D. R. Clandinin, Z. Hawrysh, J. Howell, J. A. Hanson, R. G. Christian and G. Milne

During the past year, numerous reports from various parts of the world have come to our attention concerning the laying of "fishy eggs" and the occurrence of "liver hemorrhage" in laying chickens fed rations containing rapeseed meal (RSM). Certain strains or breeds of chickens seemed to be involved more than others. In most instances, the reports have involved commercial flocks of birds with no control birds. This has made it difficult to ascertain whether RSM at recommended levels of usage was, in fact, the cause of the problem.

While up to this point in time, it has been quite well documented that levels of Canadian-type RSM in excess of 5 % of the ration may cause increased mortality in laying birds, it has not been shown conclusively that a disproportionate percentage of the mortality that occurred was caused by hemorrhage of the liver. In addition, in Canada, where the Single Comb White Leghorn (SCWL) is the principal egg layer, the laying of "fishy eggs" by birds fed rations containing RSM has not been reported.

In order to throw some light on these problems it was decided to study the possible effects of strain or breed of bird, level of inclusion of RSM in the ration and the presence of a source of myrosinase (the enzyme(s) which liberates the thyrotoxic compound(s) from the glucosinolates in RSM) in the ration on mortality caused by hemorrhage of the liver and on the laying of "fishy eggs".

Experimental

General

Three experiments were conducted. In Experiment 1, comparisons (Table 1) were made involving duplicate lots of 80 SCWL (Shaver) in floor pens fed two types of RSM at the 0, 5 and 10 % levels. One RSM was prepared from the Span variety of rapeseed, a B. campestris type, more or less typical of the type of rapeseed that has been grown in Canada during the past two years. It is moderately low in progoitrin content as compared to varieties of rapeseed grown in Europe. The other RSM was prepared from the Bronowski variety of rapeseed, which is very low in progoitrin content. In Experiment 2, the comparisons (Table 1) involved duplicate groups of 20 White Plymouth Rocks (WPR) in floor pens fed control rations and rations containing 5 or 10 % RSM prepared from B. napus type rapeseed and 3 % ground raw rapeseed. The latter was added as a source of myrosinase in order to cause the hydrolysis of the glucosinolates present in the RSM and thereby create the worst conditions possible in so far as hydroly-

TABLE 1. RATIONS[1] AND RESULTS[2]

	Experiment 1							Experiment 2					Experiment 3			
	Breed	1	2	3	4	5	6	Breed	7	8	9	10	Breed	11	12	13
Basals[3]		89.0	89.0	89.0	89.0	89.0	89.0		84.5	84.5	84.5	84.5		91.75	91.75	91.75
Ground wheat									6.4	6.4	3.2			3.5		
Wheat shorts		5.0	2.5		5.0	2.5			0.8					0.75		
Soybean meal		6.0	3.0		6.0	3.0			7.1	6.0	3.0			4.0		
Stabilized fat			.5	1.0		.5	1.0			0.1	1.3	2.5			0.25	0.25
Rapeseed oil, crude									1.2						1.2	
Rapeseed meal, Span			5.0	10.0											6.8	5.0
Rapeseed meal, Bronowski						5.0	10.0				5.0	10.0				
Rapeseed meal, *B. napus*										3.0	3.0	3.0				3.0
Ground rapeseed																
Results																
Number of birds	SCWL	320	160	160		160	160	WPR	40	40	40	40	4 Breeds	400	400	400
mg thyroid per 100 g body weight[4]	"	11.4^{a}	41.2^{b}	63.1^{c}		16.6^{a}	19.3^{a}	"	8.7^{a}	18.5^{b}	61.0^{c}	72.7^{d}	"			
Mortality, %	"	2.8^{ab}	8.1^{c}	4.9^{bc}		1.2^{a}	3.7^{abc}	"	0.0	2.5	7.5	15.0	"	7.0	12.0	6.7
Mortality due to liver hemorrhage,%	"	0.0	0.6	1.2		0.0	0.0	"		2.5	2.5	12.5	"	.25	2.75	3.0
Lipids in freeze-dried liver, %								"	17.1	19.0	15.2	15.2				
Scrambled egg, flavor	SCWL	4.9^{a}	4.8^{a}	4.8^{a}	3.9^{b5}	4.8^{a}	4.8^{a}	WPR	4.8^{a}	4.8^{a}	4.3^{b}	3.9^{c}	SCWL	4.9^{a}	4.5^{ab}	4.0^{b}
													RIR	4.9^{a}	1.7^{b}	2.2^{b}
													WPR	4.9	4.5	4.5
Scrambled egg, odor	SCWL	4.9^{a}	4.9^{a}	4.6^{b}	3.0^{c5}	4.9^{a}	4.8^{a}	WPR	4.9^{a}	4.9^{a}	4.1^{b}	3.2^{c}	SCWL	4.8	4.7	4.4
													RIR	4.9^{a}	2.7^{b}	2.9^{b}
													WPR	4.9	4.7	4.6

[1] Expressed in percentage.

[2] Value with no superscript are not significantly different. Those with a common letter in their superscripts are not significantly different, $P \leq 0.05$.

[3] Basal contained other ration ingredients needed to meet nutrient requirements of laying birds.

[4] Based on 6 birds per replicate.

[5] These were eggs from birds on the control ration that were exposed to herring meal to make them "fishy".

tic products were concerned. In Experiment 3 (Table 1), groups of 100 birds of each of four strains or breeds (Shaver SCWL, Hyline SCWL, Rhode Island Red (RIR) and WPR) in floor pens were fed a control ration, a ration containing 6.8 % RSM or one containing 5 % RSM plus 3 % ground raw rapeseed as a source of myrosinase. The birds involved in each experiment were maintained under similar conditions of housing and management.

For the purpose of this study, records were kept on mortality and complete postmortems were performed to determine the cause of deaths. Thyroid-to-body-weight ratios and the lipid contents of the livers of representative numbers of birds from each replicate in Experiment 2 were determined.

Gross and Microscopic Pathology

Gross and microscopic pathological studies were conducted on birds surviving Experiment 2 and on birds periodically sampled during the course of Experiments 1 and 3. The birds studied were killed by cervical dislocation. Special attention was given to the condition of the livers with respect to the presence or absence of hemorrhages or hematomas. An attempt was made to assess the friability of the liver and the degree of fatty infiltration of this organ on the basis of color.

Harvested tissues (see below) were fixed in 10 % buffered formalin, embedded in wax, cut at 6 microns and stained with hematoxylin and eosin (H & E). Some heart and liver tissues from Experiments 1 and 2 were fixed in 10 % buffered formalin, frozen, cut at 15 microns and stained with Oil Red 0 to permit study of the presence of lipid. A few selected liver sections were stained with Crystal Violet in an attempt to identify the nature of the pink staining hyalin-like material noted in some livers.

In Experiment 1, eight birds from each of the six treatments were killed and examined at monthly intervals from February to October, 1973. Samples of heart and liver tissues were harvested from each bird. Sections of heart and liver from all birds killed in February, May, September and October were examined microscopically following H & E staining.

In Experiment 2, twenty birds from each of the four treatments were killed in March, 1973, and heart, liver, lung, spleen, kidney, intestine, brain and peripheral nerve tissues were collected from each bird and examined microscopically after H & E staining.

In Experiment 3, four birds from each of the 12 groups were killed at monthly intervals from October, 1973 to January, 1974. Heart and liver tissues were harvested from each bird. Tissues collected in October and November, 1973 and in January, 1974 were examined microscopically after H & E staining.

In all experiments, in addition to searching for pathological changes an attempt was made to score hearts with respect to lipidosis, heterophile infiltration, monocytic infiltration and to score livers with respect to fatty metamorphosis, necrosis, hemorrhage, serum exudation and vasculitis.

Egg Odor and Flavor

The effects of treatments on egg odor and flavor were evaluated by multiple comparison tests of scrambled eggs. Panelists were trained to distinguish differences in odor and flavor during the month prior to each study. "Fishy eggs" for use in the training periods and for use as positive controls during the test periods were produced by storing negative control eggs with herring meal. The odor and flavor comparisons in each of the three experiments were conducted over periods of 10 or 12 days.

In Experiment 3, in addition to odor and flavor tests that were conducted on scrambled eggs from the Hyline SCWL, RIR and WPR on each ration treatment, the odors of 40 raw eggs from each of the groups on this experiment were independently rated by three people during October, November and December, 1973 and February and April, 1974.

Where possible, data gathered during the course of these experiments were analyzed by analysis of variance (WINER, 1971) and comparisons of means were conducted by using Duncan's new multiple range test (STEEL and TORRIE, 1960).

Results and Discussion

General

Results shown in Table 1 indicate, with respect to Experiment 1, that the Span RSM was significantly more goitrogenic than the Bronowski RSM (rations 2 and 3 vs 5 and 6). While the mortality on ration 2 was significantly higher than on other ration treatments, little attention can be attached to same since the mortality on ration 3 which contained twice as much RSM was not significantly different from that of the control groups (ration 1). Results of Experiment 2 suggest that the myrosinase present in the 3 % ground rapeseed was effective in releasing the goitrogenic compound(s) from the glucosinolates in the B. napus RSM present in rations 9 and 10. While there seemed to be a trend for increased mortality with increasing levels of RSM in the rations, the differences were not significant. With respect to mortality due to "liver hemorrhage", there also appeared to be a trend for higher incidence of "liver hemorrhage" with increased levels of RSM in the rations. However, due to the low incidence of hemorrhage, to the variability between replicates and to insufficient number of replicates, the differences were not significant at the 5 % level of probability. The data (Table 1) on the lipid contents of the livers of the birds that had been on the various treatments in Experiment 2 indicate no treatment effects.

Gross and Microscopic Pathology

Experiment 1.

Gross examination of livers for friability, fatty appearance and large or small hemorrhages showed no differences between treatments. Microscopic examination revealed some fatty infiltration between muscle bundles in some hearts from all groups. Presence of fat was confirmed by examining frozen sections stained with Oil Red 0. Only a few monocytes and heterophiles were observed in the myocardium of some hearts. Some fatty metamorphosis was observed in many livers in all groups. There were very small focal areas of necrosis in only one liver in each of three groups. Two of these were from the groups fed the control ration. A few small hemorrhages and a few small serum exudations were observed in a few livers from each group. There was some cellular infiltration around and in the walls of blood vessels in most livers in all groups. None of these findings appeared to be related to the rations fed.

Experiment 2.

Gross examination of the birds revealed no differences which could be attributed to the rations fed. Microscopic examination of lung, spleen, kidney, intestine, brain and peripheral nerve tissues showed no differences between the birds on the various ration treatments. Varying amounts of fatty infiltration between cardiac muscle bundles were present in hearts from all groups with extensive infiltration in some. Myocarditis, characterized by infiltrations of monocytes and heterophiles, was observed in many hearts from all groups with mild vasculitis observed in a relatively few cases. There did not appear to be any relationship between the observations referred to above and the rations fed. With respect to livers, there was perhaps more evidence of fatty metamorphosis in the livers from birds fed ration 7 (control ration) than from those fed the RSM containing rations, although fatty changes were found in livers from all groups. Scores for necrosis, hematomas, hemorrhage, serum exudation and cellular infiltration around vessels did not appear to be related to ration treatments. In a few livers, two from ration 9 and five from ration 10, some pink staining hyaline-like material was observed. On staining with Crystal Violet, this material did not stain with the characteristics of amyloid. It was considered that this material was coagulated protein residue from a long-standing hemorrhage or from serum exudation.

Experiment 3.

Although the microscopic examinations revealed no obvious pathological changes or differences in the hearts and livers of the different strains and breeds on the three ration treatments, some trends were noted. Very few heterophiles were observed in the hearts of birds from any of the groups. There did, however, appear to be more WPR with infiltrations of monocytes but some of this was thought to be due to leukosis. Some fatty infiltration was observed in the hearts of birds from all groups, however, the

condition appeared more marked in the RIR and WPR regardless of ration fed. Fatty infiltrations were most frequent in the livers from the WPR and least frequent in those of the Hyline SCWL. Hemorrhages, however, were more commonly seen in the livers of Hyline SCWL than in those of the other strains and breeds. Serum exudate and pink hyaline-like material were noted most often in the RIR.

Egg Odor and Taste

Experiments 1 and 2.

Results of the egg quality tests on scrambled eggs are summarized in Table 1. In Experiment 1, only the eggs that were exposed to herring meal tasted and smelled "fishy". There was an indication that the birds that received 10 % Span RSM (Ration 3), which is double the recommended level, produced eggs which smelled "fishy". The data also suggest that "fishiness" in scrambled eggs can be detected more readily by odor than by flavor. In Experiment 2, the groups which received the B. napus RSM plus ground rapeseed as a source of myrosinase, laid eggs that were definitely "fishy" in taste and odor. Here again, odor proved to be a more sensitive measure of "fishiness" than taste. The results obtained in these two experiments suggest that, at the recommended level for feeding RSM to laying chickens (5 %), Span RSM does not cause Shaver SCWL to lay "fishy eggs" but may constitute a problem with heavy-breed chickens such as WPR when a source of myrosinase is present in the ration.

Experiment 3.

The results of the taste panel work on the eggs from the Hyline SCWL, RIR and WPR fed the three different rations are summarized in Table 1. Inspection of the data indicates that flavour is not a sensitive measure of "fishiness" when low levels of "fishiness" are involved. The data also indicate that feeding rations containing 6.8 % Span RSM produced very "fishy eggs" in RIR but did not produce "fishy eggs" in Hyline SCWL or WPR. Inclusion of a source of myrosinase in the ration produced very "fishy eggs" in RIR, slightly "fishy eggs" in Hyline SCWL, and normal eggs in WPR.

The results obtained on the odor evaluations on raw eggs done periodically during the course of Experiment 3 indicate that RIR and WPR may lay appreciable percentages of "fishy eggs" on a diet containing no RSM (ration 1, Table 2). Higher percentages of such eggs, however, appeared to be laid on diets containing RSM and RSM plus a source of myrosinase (rations 2 and 3, Tables 2 and 3). The percentage of "fishy eggs" laid and the intensity of "fishy" odor decreased with length of time that the birds had been laying in the case of WPR but not in the case of RIR (Table 2). Analysis of the data collected at the five sampling periods showed non significant ration effects and significant time effects ($P \leq 0.05$). Significant breed effects for both percent "fishy eggs" and egg quality score were observed, the RIR being the worst on both counts.

Table 2: Results of raw egg odor evaluation after the birds had been on rations for two or eight months [1]

	% "Fishy Eggs" Laid						Egg Quality Score					
	2 months			8 months			2 months			8 months		
Rations	11	12	13	11	12	13	11	12	13	11	12	13
SCWL, Shaver	0	0	0	0	1	0	100	100	100	100	100	100
SCWL, Hyline	0	0	0	0	2	0	100	100	100	100	100	100
RIR	17	35	44	2	35	33	91	85	81	100	85	84
WPR	15	22	17	0	3	6	92	92	96	100	99	98

[1] Egg odor evaluations were done after the birds had been on rations for 2, 3, 4, 6 and 8 months. On each occasion 40 eggs from each of the twelve groups were rated for "fishy" odor from 1 to 5 by three people. A rating of 1 represented a "very fishy egg"; 2, a "fishy egg"; 3, a "moderately fishy egg"; 4, a "slightly fishy egg" and 5, a normal egg. % "fishy eggs", represents the percentage of eggs that inhibited any degree of "fishy" odor while, egg quality score, takes into account the degree of "fishy" odor noted. In the latter regard a rating of 100 represents normal eggs. In the above table only the initial and final evaluations are presented in order to conserve space. Statistical analyses referred to in the text, however, involved all of the data collected.

Table 3: Effects of ration and breed on raw egg odor evaluation [1]

	Rations [2]			Breeds [2]			
	11	12	13	SCWL Shaver	SCWL Hyline	RIR	WPR
% "fishy eggs"	4	11	12	0.6^{a}	0.6^{a}	25.9^{b}	8.4^{ab}
Egg quality score	99	96	96	99.9^{a}	99.9^{a}	89.7^{b}	97.3^{a}

[1] See note on Table 2.

[2] Values with a common letter in their superscripts are not significantly different $P \leq 0.05$. Rations were not significantly different for "% fishy eggs" or for "egg quality score", $P \leq 0.05$.

Summary

Studies on the possible relationship between the feeding of rations containing rapeseed meal to laying hens and the occurrence of "liver hemorrhage" and the laying of "fishy eggs" would seem to support the following:

1. Inclusion of higher than recommended levels of RSM in the ration laying hens may cause increased mortality and perhaps excessive mortality from "liver hemorrhage". However, in the studies conducted, no microscopic pathological evidence was obtained which suggested that levels of RSM up to 10 % of the ration caused abnormalities in the tissues examined. (It is, of course, acknowledged that such levels of RSM cause histological changes in thyroid tissue).

2. Inclusion of 6.8 % RSM in the ration of laying hens caused no adverse effects on egg odor or taste in eggs from SCWL or WPR but caused the laying of "fishy eggs" in RIR. The presence of a source of myrosinase in laying rations containing RSM and the inclusion of high levels of RSM in laying rations may be expected to increase the "fishy egg" problem.

In view of these findings it is recommended that for the production of market eggs levels of RSM in excess of 5 % should not be fed to SCWL and that no RSM should be included in the ration of brown-egg layers that are used for the production of market eggs. In addition, exogenous sources of myrosinase, such as grain contaminated with mustard seed or rapeseed, should not be permitted to get into rations containing RSM.

Acknowledgements

The assistance of Dr. R. T. Hardin, Associate Professor of Poultry Genetics, Department of Animal Science, University of Alberta in connection with the statistical analysis of the data and of members of the taste and smell panels was greatly appreciated. The study was supported, in part, by grants from the National Research Council of Canada, the Alberta Agricultural Research Trust and the Rapeseed Association of Canada.

References

1. STEEL, R. G. D. and J. H. TORRIE (1960): Principles and Procedures of Statistics. McGraw-Hill, New York

2. WINER, B. G. (1971): Statistical Principles in Experimental Design. McGraw-Hill, New York