Effect of Riboflavin Intake on Egg Production and Riboflavin Content of Eggs*

CHARLIE F. PETERSEN, C. E. LAMPMAN, AND OLOF E. STAMBERG[1]
Departments of Poultry Husbandry and Agricultural Chemistry, University of Idaho, Moscow

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IT WAS reported by Norris and associates (1936) that a ration containing 130 ug riboflavin per 100 grams was as effective in promoting egg production as a similar ration to which was added 5.0 per cent dried whey and that riboflavin in relation to egg production was not a limiting factor. Davis, Norris, and Heuser (1938) obtained normal production with a ration computed by them to contain 100 Cornell units per 100 grams. The work of Lepkovsky and associates (1938) indicates that these values might be low for normal production during all seasons of the year. These workers fed a ration which computed 164 ug riboflavin per 100 grams calculated from average figures given by the National Research Council (1944), and it did not promote maximum egg production during the winter months of December, January, and February. The same ration plus riboflavin resulted in greatly increased egg production during this period. An explanation for this seasonal drop in production might be that hens had obtained added riboflavin through coprophagy, but that adverse temperature prevented any large increase of the riboflavin content of the fecal matter during the winter months. The work of Hunt, Winter, and Bethke (1939) showed that a ration which analyzed 80 ug per 100 grams resulted in a complete cessation of egg production when the experimental birds were confined to batteries. The conflicting data would indicate that the actual riboflavin requirements of hens for normal egg production has not been definitely established.

Of still greater interest is the marked variation of the riboflavin content of experimental rations as reported by various workers. Much of the early data were obtained with rations which were found to contain approximately 1.0 ug riboflavin per gram of feed. A review of the literature shows that with the use of improved microbiological and chemical techniques of analyses, increased values of as much as 50 percent have been found for feed ingredients of low riboflavin content which compose a large percentage of the experimental rations. In addition, it has been shown that the riboflavin content of individual feeds varies considerably. Vitamin-free casein, which has been used extensively as a protein supplement, has been shown by Cannon *et al.* (1945) to contain appreciable amounts of riboflavin, especially for use in a basal ration of controlled experimental work in relation to riboflavin requirements. This variation was also found in this study in

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[1] Now with the Red Star Yeast & Products Company, Milwaukee, Wisconsin.

routine analyses of feed ingredients. The riboflavin content of the vitamin-free casein varied from 0.00 to 1.60 ug/gm, with an average of approximately 1.1 ug/gm for 13 samples. Wheat samples analyzed by Conner and Straub (1941) varied from 0.81 to 1.91 ug/gm, and values obtained by other workers also showed extreme variation for various feed ingredients. In contrast to rations which were found to contain approximately 1.0 ug/gm, similar experimental rations containing cereal grains and vitamin-free casein were reported more recently by Engel, Phillips, and Halpin (1940) and Culton and Bird (1940) to contain by analysis 1.60 and 1.75 ug/gm, respectively. The experimental ration used in this study analyzed 1.27 ug/gm—and when the added 18 percent dextrose is considered, the ration, undiluted, would analyze near these higher values.

It has been shown by numerous workers in recent years that within certain limits there is a direct correlation between the riboflavin content of the feed fed to hens and the amount of riboflavin contained in eggs produced. Bethke, Record, and Wilder (1936) showed a direct correlation of the riboflavin content of the eggs produced and the ration fed hens by feeding dried eggs to chicks and rats. Lepkovsky and associates (1938) also reported similar results by the chick-growth method. Norris and Bauernfeind (1940) made a thorough study of the effect of varying levels of intake upon both the rate of change and the concentration of riboflavin in eggs produced. It was found by chemical analysis that a period of 2 to 4 weeks was required to obtain maximum change in egg content when birds were transferred from a high to low or low to high riboflavin intake. It was also reported by these workers that there was no additional increase in the riboflavin content of the egg when the ration contained from 800 to 1,000 ug per 100 grams. Stamberg, Petersen, and Lampman (1946) found that maximum change in egg content was obtained in 6 to 8 days when birds in batteries were transferred from a low to high and a high to low riboflavin content ration. This was determined from an analysis of individual eggs and internal yolks of several hens. It was also observed that hens confined to batteries produced eggs with a maximum riboflavin content when fed a ration containing 750 ug per 100 grams. Little or no increase was obtained from the individual hens when they were transferred to rations containing 1,500 and 2,600 ug per 100 grams

TABLE 1.—*Composition of basal ration*

Ingredient	Percent
Wheat bran	5.00
Ground oats	20.00
Ground barley	20.00
Ground corn	20.00
Vitamin-free casein	10.00
Dextrose	18.00
Oyster shell flour	2.00
Bone meal	4.00
Iodized salt	0.50
400D	
Vitamin A and D oil (2,000A)	0.50
$MnSO_4$ (10 gms/cwt)	
Vitamin additions*	

* Vitamin additions to experimental ration (milligrams per 100 grams).

2-methyl 1–4 naphthoquinone	0.2
Thiamin (hydrochloride)	0.2
Pyridoxine (hydrochloride)	0.3
Alpha tocopherol	1.0
Calcium pantothenate	1.5
Niacin	1.8
P-amino-benzoic acid	10.0
Inositol	40.0
Choline chloride	150.0

The study reported here concerns the effect of feeding several levels of riboflavin upon egg production and riboflavin content of eggs when birds were confined to individual laying cages in order to prevent birds having any access to riboflavin other than the experimental diet. Hatchability and subsequent chick growth

response were also obtained and will be reported in a later report.

EXPERIMENTAL

Six groups of eight Single Comb White Leghorn pullets were confined to individual wire-bottom laying cages at approximately 6 months of age. The birds were fed a mash which contained 16.0 per-

TABLE 2.—*Percent egg production for each group by bi-weekly periods for 4 weeks preceding and 17 weeks feeding of experimental rations*

Ration	Control—300 ug. per 100 grams		Results while feeding experimental regions								Av. for period
Period	Oct. 16–31	Nov. 1–15	Nov. 16–30	Dec. 1–15	Dec. 16–31	Jan. 1–15	Jan. 16–31	Feb. 1–14	Feb. 15–28	Mar. 1–15	
Group no. 1	76.7	82.5	64.5	18.3	1.7	—	—	—	2.6	6.1	11.56
Group no. 2	64.5	72.5	55.0	54.2	59.2	65.8	74.0	67.0	65.2	70.5	63.96
Group no. 3	60.8	75.8	62.5	63.3	56.7	52.4	70.5	62.3	67.4	65.3	62.38
Group no. 4	44.2	64.2	64.2	54.2	63.3	50.8	55.8	54.5	60.7	69.7	59.17
Group no. 5	56.7	74.2	68.3	68.2	76.7	65.8	70.0	51.8	52.7	54.5	63.85
Group no. 6	63.3	66.7	60.8	66.7	74.1	68.3	65.8	67.9	67.0	73.3	68.02

cent protein and which analyzed 3.20 ug riboflavin per gram. After a period of three weeks, several eggs from each hen were analyzed for riboflavin, and the hens were then redistributed so that the riboflavin content of composite samples of eggs from each group were approximately equal. The experimental feeding was begun after four weeks of confinement and continued for seventeen weeks. The basal experimental ration was composed of ground barley, oats and wheat, wheat bran, vitamin-free casein, minerals, dextrose, and various vitamin additions, as shown in Table 1. The basal diet analyzed 1.25 ug per gram, as determined by analysis of the individual feed ingredients, and sufficient synthetic riboflavin was added to the other groups to give approximate levels of 2.50, 3.60, 5.00, 7.50, and 10.00 ug per gram. Analyses of the completed rations showed that all were within ± 0.11 ug of the desired values. Batteries and water equipment were cleaned regularly to prevent the birds obtaining any other source of riboflavin and precautions were taken to prevent any spillage of feed from one group to another. An auxiliary heat unit was provided in the battery room to maintain a temperature of from 45 to 48°F.

All eggs produced were recorded and marked with the hen number and date. Riboflavin analyses were made of composite samples of all eggs from each group for each two-day period for the first six weeks of experimental feeding and for one week during the 15th week of experimental feeding. Body weights were recorded at the beginning and completion of the experiment, and feed consumption for each group for the experimental period was recorded.

The riboflavin content of the eggs was determined by the method of Peterson, Dearstyne, Comstock, and Weldon (1945). The riboflavin determinations were made with a Coleman fluorophotometer.

RESULTS AND DISCUSSION

The basal ration which analyzed 1.27 ug riboflavin per gram was not adequate for body maintenance or egg production when fed to birds confined to laying batteries. The eight pullets in this group were laying at the rate of 76 to 82 percent for the four weeks preceding the experimental feeding. Within two weeks of experimental feeding, six of the eight hens

had ceased laying and the remaining two went out of production during the fourth and fifth weeks. Two hens produced an occasional egg again during the fifteenth to seventeenth week. The data for egg production for each group by bi-weekly periods are given in Table 2. There did not appear to have been any deficiencies in other groups, either riboflavin or other factors. All groups which received intake levels from 2.50 to 10.00 ug per gram continued to lay at a very high rate for the 17-week experimental period, averaging from 59.17 to 68.02 percent. The 2.50 ug level (group 2) was approximately intermediate between these two percentages, averaging 63.96 percent for the period.

TABLE 3.—*Data by groups on body weight, feed consumation, and riboflavin utilization*

Group no.	1	2	3	4	5	6
Riboflavin content of feed (ug/gm.)	1.27	2.54	3.62	5.11	7.49	10.00
Av. body wt.—start lb.	3.84	3.90	3.90	3.88	4.00	3.77
Av. body wt.—finish lb.	2.76	4.19	4.45	4.37	4.53	4.33
Feed consumed per bird for period (lb.)	15.80	30.20	32.30	29.50	31.80	31.50
Feed consumed per bird per day (grams)	60.30	115.40	123.20	112.90	121.30	120.00
Riboflavin consumed per bird per day (ug)	90.40	288.50	443.50	564.50	909.80	1200.00
Av. riboflavin content of egg (ug per gm)	1.79	2.36	4.60	5.36	5.51	6.05
Av. egg wt. (total egg less shell) grams	47.40	52.40	49.80	49.50	50.60	46.20
Av. total riboflavin content per egg (ug)	84.85	123.66	229.08	265.32	278.81	279.51
Percent riboflavin intake transferred to eggs	10.86	27.41	39.24	27.81	19.57	15.84

The data in Table 3 show that the birds in group 1 lost an average of 1.08 pounds during the experimental period. All birds in this group went into a partial or complete molt and remained in very poor condition. As noted in the same table, the feed consumption and, consequently, the riboflavin intake was greatly reduced for this group. The average feed consumption per bird per day (60.3 grams) was approximately one-half that consumed by birds receiving the higher levels. With this reduced feed intake the birds consumed an average of only 90 ug riboflavin per day, whereas the birds receiving 2.50 ug per gram of ration actually consumed three times as much riboflavin. It would appear from this data that the actual riboflavin requirements for body maintenance and maximum egg production was more than 1.27 ug per gram of ration. Had the birds had access to other sources of riboflavin, such as is normally present in fecal matter, this lower level might have sufficed. Also of interest is the fact that the average body gain for group 2, receiving 2.50 ug per gram, was only 0.29 pound during the period, compared to better than 0.50 pound for the higher intake groups. This would not appear to be marginal but, when the riboflavin storage of body tissue is also considered, it was found that there was not a maximum storage of riboflavin in the white meat and livers of this group of hens, Stamberg, *et al.* (1947), indicating a sub-marginal intake for maximum body maintenance of hens in high production.

The effect of the various riboflavin intake levels fed upon the riboflavin content of eggs produced is tabulated in Tables 3 and 4 and shown graphically in

Figures 1 and 2. Table 3 gives the average riboflavin concentration of all eggs produced from each group, the average total riboflavin content of each egg produced, and the percentage of riboflavin consumed by each group of hens which was transferred to the eggs. Table 4 gives the average values obtained for all eggs produced based upon an aliquot sample of all eggs layed for each two-day period. These values are given for each group for two weeks preceding the experimental feeding. The first eight days of experimental feeding are also averaged separately from the remaining experimental period, inasmuch as previous work, Stamberg *et al.* (1946), had shown that it required that time interval for the riboflavin content of eggs to be adjusted when birds were transferred from one intake level to another.

The riboflavin content of the eggs of the birds in group 1 decreased markedly while the birds were in production. This is shown both in Table 4, showing the actual analytical values, and graphically in Figure 1. The average concentration of riboflavin of all eggs during the pre-experimental period was 3.32 ug riboflavin per gram of fresh egg as compared to 1.79 ug per gram for the experimental period.

The birds receiving 2.54 ug per gram of ration (group 2) also produced eggs of lower riboflavin content on the experimental ration than during the preceding

TABLE 4.—*Summary of riboflavin content of eggs from hens fed varying levels of riboflavin (micrograms per gram fresh egg)*

Dates	Ration	Groups					
		1	2	3	4	5	6
Nov. 2–3	Control	2.73	2.85	3.30	2.72	2.95	2.98
4–5		3.49	3.87	3.19	3.76	3.97	2.98
6–7		3.63	3.58	3.30	2.62	3.19	3.40
8–9		3.10	3.90	3.78	3.53	3.61	3.19
10–11		3.20	3.12	2.90	3.59	2.90	3.36
12–13		3.49	3.01	3.50	2.82	3.01	2.85
14–15		3.51	3.21	3.29	3.25	3.10	3.37
Av. for pre-exp. ration		3.32	3.35	3.31	3.32	3.25	3.29
Nov. 16–17	Experimental ration	2.83	3.08	3.68	3.55	3.89	3.73
18–19		2.69	3.02	3.62	3.88	4.50	4.96
20–21		2.61	2.73	3.92	4.12	5.15	5.49
22–23		2.06	2.62	4.18	5.69	5.81	6.91
Av. for 1st 8 days on exp. rations		2.53	2.83	3.85	4.31	4.84	5.84
Nov. 24–25		2.00	2.90	4.51	5.60	5.64	6.09
26–27		2.19	2.83	5.28	6.05	5.95	6.73
28–29		2.06	2.63	4.68	5.34	5.96	6.38
Dec. 30–1		1.38	2.53	4.77	5.31	5.65	5.83
2–3		1.63	2.49	4.75	5.63	5.76	5.83
4–5		1.57	2.29	4.57	4.73	4.89	5.95
6–7		1.62	2.21	4.53	4.58	5.64	6.26
8–9		1.34	2.67	4.49	5.90	5.36	6.44
10–11		1.70	2.06	4.53	5.38	5.66	5.86
12–13		1.67	2.37	4.59	5.31	5.71	5.96
14–15		1.79	2.24	4.53	5.51	5.35	6.07
16–17		1.30	2.02	4.52	5.59	5.64	5.90
18–19		1.43	1.98	4.50	5.37	5.51	5.46
20–21		0.00	2.00	4.49	5.50	5.50	6.16
22–23		0.00	1.97	4.27	5.19	4.96	5.81
24–25		0.00	2.08	4.47	4.96	5.13	6.11
Feb. 14–15		0.00	2.44	4.68	5.38	5.19	6.10
Ac. for experimental period		1.79	2.36	4.60	5.36	5.51	6.05

period when they received a ration containing 3.20 ug per gram. The riboflavin content of eggs was considerably higher from group 3 which was fed a level of 3.62 ug per gram; these eggs containing an average of 4.60 ug per gram of egg. The maximum concentration of riboflavin in the egg was obtained from group 4, which received a ration containing 5.11 ug per gram. Table 4 and Figure 1 show that the 5.11 and 7.49 ug per gram intake levels produced eggs of almost equal content (5.36 and 5.51 ug/gm), whereas group 6, receiving 10.00 ug per gram, produced eggs of slightly higher content. It is noted in Table 3 that this group of hens layed eggs considerably smaller than the average and, when the average riboflavin content of the total egg was computed, it was found that groups 4, 5, and 6 produced eggs with an average riboflavin content of 265.32, 278.81, and 279.51 ug per egg, which indicates that an intake level of 5.00 ug per gram would produce eggs of maximum or near maximum riboflavin content. This level was also found to be necessary for these same hens to give maximum body storage when in heavy production.

Another point of interest in Table 3 is the percentage utilization of riboflavin obtained on the various intake levels. Birds receiving 3.62 ug per gram of ration converted 30.24 percent to the eggs. The two intake levels of 2.54 and 5.11 were approximately equal (27.41 and 27.81 percent) and very close to the most efficient level. The two higher intake levels were much more inefficient, transferring only 19.57 and 15.84 percent for the 7.49 and 10.00 ug levels, respectively. This level of maximum utilization which gives maximum egg content is also shown by the curve in Figure 2. It is noted here that the curve tends to level off at a feed intake level of 5.00 ug per gram.

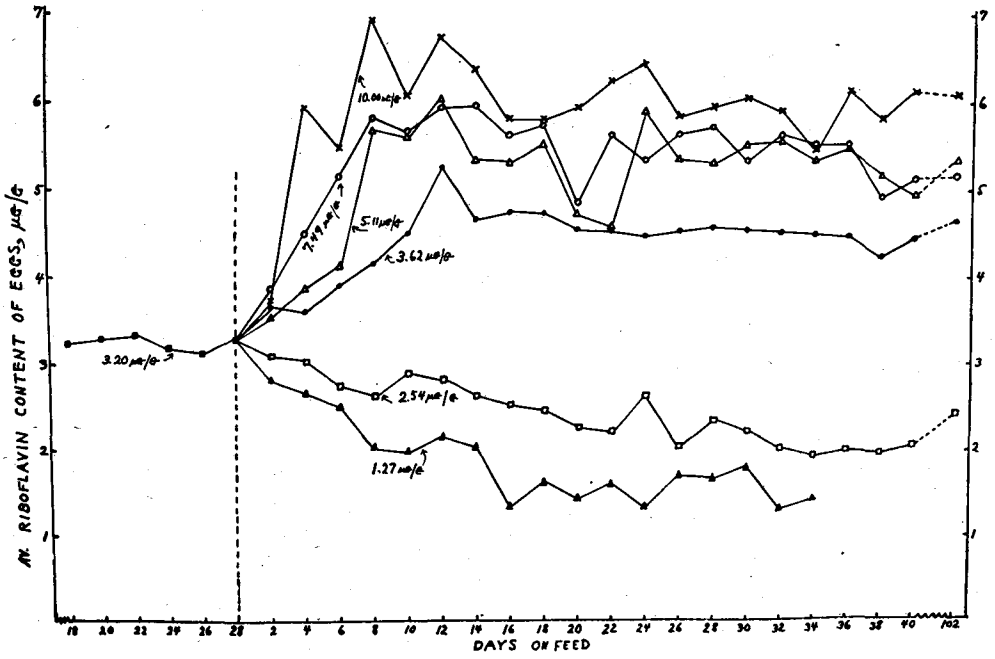

FIG. 1. Effect of intake of riboflavin on riboflavin content of eggs (six intake levels).

SUMMARY

Six groups of eight hens each were confined to laying batteries and fed an experimental ration in which the riboflavin content varied from 1.27 to 10.00 ug per gram. Body weight, feed consumption, and egg production records were obtained. All eggs produced during the first six weeks and the fifteenth week of experimental feeding were analyzed for riboflavin, using a Coleman fluorophotometer.

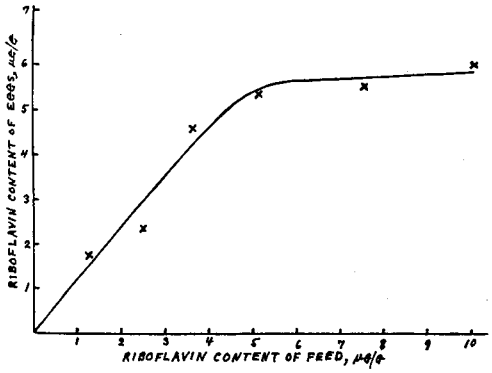

FIG. 2. Riboflavin content of eggs in relation to feed content.

A ration containing 1.27 ug per gram of riboflavin was not adequate for maintenance of body weight, egg production, or adequate riboflavin content of eggs produced.

Maximum body weight was not maintained on the group receiving 2.5 ug per gram, although egg production was normal.

Maximum transfer of riboflavin to the eggs occurred when the birds received 5.11 ug or more per gram of ration. The hens which were fed 3.62 ug/gm were most efficient in transferring riboflavin to the eggs, followed closely by the 2.54 and 5.11 ug levels.

It would appear that an intake level between 3.60 and 5.11 would result in eggs of maximum riboflavin content and permit birds to carry a maximum body reserve.

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