THE UTILIZATION OF LACTOSE BY THE CHICKEN[1]

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The value of milk, in its various forms, as a poultry feed has been clearly established, the high quality of its proteins and minerals being its chief assets. The present investigation was undertaken primarily for the purpose of determining whether or not lactose, the carbohydrate of milk, was utilized by the chicken. It is not always realized that lactose represents approximately 38 per cent of the total solids and approximately 30 per cent of the energy of whole milk, while the total proteins contain but 26 per cent of the solids and approximately 20 per cent of the energy of whole milk. In some commercial milk preparations the percentage of lactose is much higher; whole milk powder contains 34 to 41 per cent, skim milk powder 48 to 54 per cent, and secwa, or dried whey, contains approximately 74.5 per cent lactose and 14.2 per cent soluble albumin (*5*).[3] From a practical point of view the carbohydrates of a ration usually seem of less importance than the protein because of the lower relative cost of the former, but a knowledge of the amount and utilization of the carbohydrates present is necessary in order to formulate the most desirable and economical ration for a particular purpose. Thus a knowledge of the amount and degree of utilization of the lactose in milk is of practical importance to the science of poultry feeding. The question is also of theoretical interest because at no stage in the life of the chicken is milk, or any other substance containing lactose, a part of its natural diet, and therefore the utilization of lactose, which normally requires the presence of a lactase, might rightfully be questioned on teleological grounds.

The literature available on the subject of the utilization of lactose by the fowl is very meager. In addition to investigations on the adaptation of various digestive organs to lactose, only two studies have been found dealing directly with the question. Shaw (*7*), in a study of digestion in the chick, concluded that lactose was not digested and, further, that it acted as an irritant to the gastrointestinal mucosa. Chicks fed from birth on milk alone died on the third day and the contents of no part of the intestinal tract gave a positive test for monosaccharids with Barfoed's reagent. That lactose was present was shown by preparing the phenyllactosazone crystals. Plimmer and Rosedale (*6*), on the other hand, claim to have fed chickens from birth to a period exceeding three months on a diet containing lactose. Failure to find reducing sugars in the excreta was taken to indicate the assimilation of lactose.

EXPERIMENTAL DATA

In determining the utilization of lactose, the reducing sugars present in the excreta of hens fed on an ordinary cereal diet and on a diet containing variable amounts of lactose were estimated. Seven experiments,

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[3] Reference is made by number (italic) to "Literature cited," p. 604.

each of one week's duration, were made. In all experiments in which lactose was fed, except Experiment No. 2, the hens received the lactose with their mash on each of three successive days. The excreta were collected daily during these three days as well as during the four following days in order to allow sufficient time for the excretion of any unabsorbed lactose. Between successive experiments four days were ordinarily allowed to elapse.

In Experiment No. 2 two grams of lactose were fed daily to each hen for seven days. The excreta were collected on the seventh day only. Four mature White Wyandotte hens were used in this investigation. Each hen was kept in a wire cage 15 inches wide, 24 inches long, and 14 inches high. The wires of the cage were 2 inches apart one way and 4 inches apart the other. In the bottom of the cage was the excreta-collection pan, which was of the same width and length as the cage and 1.5 inches deep. It rested on flat iron grooves which held the pan firmly in place but which permitted removal for cleaning. The pan was made water-tight, of medium weight galvanized iron. Inside of the pan was an iron frame covered with hardware cloth. This frame was just enough smaller than the pan to fit closely within it and was raised about 1 inch from the bottom. The hen stood on this frame and the excreta for the most part soon passed through the hardware cloth into the pan below. In this manner the excreta were neither trampled over nor scratched out and lost. The feeding and watering jars were supported outside the cages and protected in such a manner that the hen was unable to scatter feed into the collection pan. The hens seemed contented and appeared not to mind in the least the comparatively close quarters in which they were confined.

During each experiment the collection pans were cleaned daily. An extra collection pan was available and was substituted for the regular pan while the latter was being cleaned. In cleaning the pan, the frame was first freed from any excreta remaining on it by scraping the excreta into the pan below. The pan itself was then thoroughly cleaned with a steel spatula. The excreted material from each hen was kept at 0° to 4° C. in an air-tight, half-gallon glass fruit jar until the end of the experiment, when it was analyzed. In order to preserve the excreta, an equal mixture of alcohol and water, containing 10 per cent thymol, was poured over the frame each day after cleaning, the excess being caught in the pan. After running the solution over all parts of the pan, the excess was drained into the jar containing the excreta. A small amount of powdered thymol was also sprinkled over the excreta in the jars.

The procedure adopted in this investigation depended upon the fact that the excreta from chickens fed on a cereal diet normally contain little, if any, reducing sugars. This was shown by Brown (*3*) who carefully examined, by the phenyl-hydrazine test, the excreta of chickens fed on a corn diet. No trace of an osazone could be detected. Plimmer and Rosedale (*6*) found no reducing sugars in the excreta of chickens even when fed on a diet containing lactose. In the present investigation the results obtained indicate that there is no constant excretion of reducing sugars in chickens fed on a cereal diet. The determination of the completeness of utilization of ingested lactose, therefore, consisted in the analysis of the excreta for reducing sugars. In all cases the quantitative analysis was substantiated by a qualitative examination of the excreta for sugars by the formation of osazones and their identification under the microscope.

The procedure adopted for the extraction of the reducing substances in the excreta was as follows: The total excreta of each hen for the period of the experiment were extracted by pouring 200 to 500 cc. portions of hot water over the excreta in a large evaporating dish, macerating thoroughly with a pestle, and filtering through cloth. The residue in the cloth was returned to the dish and extracted again. When the total volume of the washings measured 4,000 cc., the extraction was considered complete. An extract of this volume was previously shown to be sufficient by mixing 25 gm. of lactose and 25 gm. of glucose with a seven-day collection of excreta and extracting with 200 to 500 cc. portions of hot water until the last extract failed to give a carbohydrate test with Fehling's solution. The reducing substances in the total excreta for the period of the experiment were estimated by determining the reducing substances, calculated as lactose, $C_{12}H_{22}O_{11}$, in aliquots of the thoroughly mixed extract.

Two methods were used throughout for the determination of reducing substances. The first was the combination of the Munson and Walker and the Bertrand (*2*) methods, as described by Mathews (*4, p. 994*), for the quantitative determination of reducing sugars in urine and other fluids, and the second was Benedict's (*1*) method for the estimation of glucose in urine. The first is a method of the Association of Official Agricultural Chemists [4] for the determination of reducing sugars in foods and feeding stuffs and has been found in this laboratory to be rapid and accurate. This method, however, has the disadvantage that substances other than sugars, uric acid in particular, are capable of effecting reduction of the alkaline cupric tartrate and hence, if present, would be calculated as lactose. Uric acid would, of course, be present in considerable quantities in hot aqueous extracts of chicken excreta. However, since the quantity of uric acid excreted daily from a diet in which only the amount of lactose varied would probably change but slightly, it was thought that the variation in quantity of reducing sugars present could be estimated without the previous removal of uric acid. On the other hand, the Benedict method is not appreciably affected by the presence of uric acid. Tests designed to show the effect of uric acid and to determine the applicability of the combined Munson-Walker-Bertrand and the Benedict methods to aqueous extracts of chicken excreta containing a constant amount of uric acid and varying amounts of lactose were conducted.

First, the effect of uric acid on the determination of lactose in milk by each of the two methods was tested. Lactose was determined in a sample of separated milk by the two methods in the usual manner. Then, uric acid, varying from 0.01 gm. to 1 gm. was added to 25 cc. portions of the sample and the lactose was determined by the combined Munson-Walker-Bertrand and by the Benedict methods in the usual manner with the following averaged results.

[4] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to November 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

TABLE I.—*Effect of uric acid on the determination of lactose by the Munson-Walker-Bertrand and the Benedict methods*

PER CENT OF LACTOSE IN SEPARATED MILK

Uric acid added.	Munson-Walker-Bertrand method.	Benedict method.
Gm.		
.....	4. 145	4. 152
.....	4. 106	4. 156
.....	4. 176	.....
.....	4. 192	.....
	[a] 4. 155	[a] 4. 154
0. 01	4. 200	4. 157
0. 10	4. 400	4. 150
0. 90	5. 475	4. 161
1. 00	.....	4. 173

[a] Average.

It is readily seen from Table I that uric acid markedly affects the estimation of lactose by the combined Munson-Walker-Bertrand method. In fact, where 1 gm. of uric acid was added to a 25 cc. portion of separated milk it was impossible to carry out the test. That the Benedict method is unaffected by uric acid to any appreciable extent is also shown.

In a second test about 1,200 gm. of combined excreta from four hens, fed on the standard cereal diet, were mixed thoroughly and four portions of 200 gm. each removed. To each of two portions, 25 gm. of lactose were added and the samples mixed. All four samples were then extracted in the same manner with four 300 cc. portions of hot water. Each extract was made up to 1,500 cc., mixed, and lactose determined in aliquot portions by both sugar methods in the manner in which they are used in urine analysis. The results have been averaged and summarized in Table II. Table II also contains the averaged results obtained when 1 and 2 gm. of lactose were added to excreta in a manner similar to that when 25 gm. were added. The reducing substances were determined by the Benedict method only in these cases, however.

TABLE II.—*Recovery of lactose added to excreta*

Sample of lactose.	Total reducing substances calculated as lactose.			
	Munson-Walker-Bertrand method.		Benedict method.	
	I.	II.	I.	II.
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
0	0. 34	0. 35	0. 03	0. 04
25	25. 81	26. 21	24. 86	25. 13
1	(a)	(a)	1. 08	1. 11
2	(a)	(a)	2. 30	2. 21

[a] Not determined.

These tests indicated that for the purpose of this investigation either method could be used with a fair degree of accuracy. Both methods were employed throughout and the technique used was the same as that recommended for each method when applied to urine analysis. In order to obtain additional data and also to serve as a check on the quantitative results, the phenyl-hydrazine test was uniformly applied to all extracts. The osazones were examined microscopically only.

TABLE III.—*Summary of results on the utilization of lactose by the hen*

Experiment No.	Daily diet.	Hen No.	Duration of experiment.	Presence of osazones in excreta.		Total reducing substances excreted calculated as lactose, $C_{12}H_{22}O_{11}$.	
				Lactosazone.	Glucosazone.	Munson-Walker-Bertrand method.	Benedict method.
			Days.			*Gm.*	*Gm.*
I	Basal ration plus 6 gm. lactose	1	.....	—	—	1. 98	1. 70
		2	7	—	—	0. 80	0. 64
		3	.....	—	—	1. 21	0. 78
		4	.....	—	—	1. 57	1. 84
II	Basal ration plus 2 gm. lactose	1	.....	—	—	0. 00	0. 16
		2	1	—	—	0. 00	0. 03
		3	.....	—	—	0. 00	0. 03
		4	.....	—	—	0. 00	0. 15
III	Basal ration.....	1	.....	—	—	4. 34	1. 34
		2	7	—	—	3. 29	1. 40
		3	.....	—	—	0. 63	0. 60
		4	.....	—	—	1. 54	0. 72
IV	Basal ration plus 18 gm. lactose.	1	.....	—	+	5. 52	3. 89
		2	7	—	+	6. 08	3. 78
		3	.....	+	+	5. 16	4. 08
		4	.....	+	+	10. 84	7. 78
V	Basal ration.....	1	.....	—	+	3. 08	0. 56
		2	7	—	+	2. 03	1. 33
		3	.....	—	+	3. 71	1. 68
		4	.....	—	+	2. 52	1. 05
VI	Basal ration plus 24 gm. lactose.	1	.....	+	+	3. 08	1. 79
		2	7	+	+	8. 16	5. 36
		3	.....	+	+	6. 11	3. 52
		4	.....	+	+	9. 37	7. 07
VII	Basal ration.....	1	.....	—	+	2. 38	0. 70
		2	7	—	+	3. 01	1. 26
		3	.....	—	+	2. 73	0. 98
		4	.....	—	+	3. 15	1. 61

DISCUSSION

The basal ration consisted of 30 gm. of a mixture of oats and cracked corn, morning and evening, and 30 gm. of moistened mash at noon. The mash consisted of equal parts by weight of wheat bran, flour middlings, ground corn, ground oats, and tankage. The lactose fed during all the experiments was Merck's U. S. P. lactose monohydrate which proved

to be 99 per cent pure by both the Munson-Walker-Bertrand method and the Benedict method. It was fed with the mash at noon. The results are reported in grams of lactose excreted during the experiment. As would be expected from the fact that the determination of reducing sugars is affected by uric acid in the Munson-Walker-Bertrand method, while this acid does not affect, appreciably, the Benedict method, the results obtained by the latter method are, with few exceptions, much lower than those obtained by the former. On a comparative basis, however, the differences between experiments are as well shown by the one method as by the other. In the preparation of the osazones for the identification of individual sugars in the extracts the utmost care was taken, for this method of distinguishing between the sugars has never proved to be infallible in this laboratory, and especially was this found to be the case with extracts such as those dealt with in this investigation. The purified free base was used since it was found to give more satisfactory results than the hydrochlorid. While the qualitative osazone tests were not quite as satisfactory as might be desired, they confirm very well the quantitative data. In general, they indicate that lactose does not appear in the excreta of hens until comparatively large amounts are fed, that the glucosazone usually is found whenever the lactosazone is present, and that glucose persists in the excreta for some time after the lactose has disappeared.

In feeding lactose to hens it was immediately noticed that amounts exceeding 2 gm. daily caused diarrhea, a finding in agreement with that of Shaw (7). In order to test the possibility of the diarrhea itself being the cause of an increase in the excretion of reducing substances, two experiments, in which diarrhea was caused by feeding Epsom salts ($MgSO_4 . 7H_2O$) in the drinking water, were made. The results indicated clearly that the diarrhea was not the cause of the excretion of comparatively large amounts of reducing substances when lactose exceeding 2 gm. was fed.

The results of seven experiments, summarized in Table III, show that pure lactose when added to a cereal diet is utilized to a large extent by hens. All hens excreted more or less reducing substances when fed a normal cereal diet; this was, of course, expected. The amounts of reducing substances excreted by the four hens in three seven-day experiments on the lactose-free diet varied from 0.56 gm. (calculated as lactose) to 1.68 gm. by the Benedict method, as indicated in the following summary:

Hen No.	Experiment.			Average.
	III.	V.	VII.	
	Gm.	*Gm.*	*Gm.*	*Gm.*
1	1.34	0.56	0.70	0.87
2	1.40	1.33	1.26	1.33
3	0.60	1.68	0.98	1.09
4	0.72	1.05	1.61	1.13
Average				1.10

Since the method of extraction was always the same, that is, the number of extractions and the quantity and temperature of the water were exactly the same in all cases, it follows that the amounts of reducing substances excreted by each hen during the different weekly periods on the ordinary cereal diet were not constant. The average amount of reducing substances excreted by each hen during seven days was, by the Benedict method, 1.10 gm., and by the Munson-Walker-Bertrand method, 2.70 gm., expressed as lactose. If, therefore, we use these average figures as representing the grams of reducing substances originating from the basal ration in the extract of any seven-day collection of excreta, the percentages of lactose absorbed, when varying quantities of lactose were fed, may be calculated as shown in Table IV.

TABLE IV.—*Percentage absorption of lactose*

Hen No.	Percentage of lactose absorbed when the following quantities were fed—							
	2 gm.		6 gm.		18 gm.		24 gm.	
	Benedict method.	Munson-Walker-Bertrand method.	Benedict method.	Munson-Walker-Bertrand method.	Benedict method.	Munson-Walker-Bertrand method.	Benedict method.	Munson-Walker-Bertrand method.
1	100	100	90	100	85	85	97	98
2	100	100	100	100	85	82	83	77
3	100	100	100	100	84	87	90	86
4	100	100	88	100	63	55	75	73

Table IV is self-explanatory; it shows that hens will utilize lactose up to 8 gm. daily fairly completely. Attempts to feed larger quantities failed because the hens would not voluntarily eat their mash when more than 8 gm. had been mixed with it. Even when 8 gm. were fed daily it was necessary to force-feed much of the mash on the third day to all hens. This was probably due to the severe diarrhea caused by this quantity of lactose when fed in this form. Because of the severe diarrhea it was not considered wise to attempt to feed larger amounts.

The difficulties experienced in these experiments in inducing the chickens to consume large quantities of pure lactose when mixed with the feed, and the severe diarrhea always obtained when even 8 gm. a day were consumed, are in striking contrast to the experience of Plimmer and Rosedale (5). These investigators fed a diet containing secwa (a dried whey preparation containing 74.5 per cent lactose) to chickens from birth for a period exceeding four months. The amount of secwa in the diet was usually 25 per cent but in some cases was even higher. The quantity of lactose consumed in this form by each bird was as high as 22 gm. daily. Good gains were obtained and the health of the birds was excellent throughout the experiment. No reasonable explanation of these differences occurs to us.

SUMMARY

Lactose, up to 8 gm. per hen per day, was utilized fairly completely, thus making it evident that the lactose present in the quantity of whole milk, skim milk, whey, or buttermilk normally consumed (100 to 200 cc.) by chickens would be completely absorbed.

When lactose appears in the excreta, it is usually accompanied by glucose.

Lactose when fed mixed with a moist mash acts as an irritant to the gastrointestinal mucosa. Chickens will not voluntarily consume more than about 8 gm. of lactose daily when fed in the form of pure lactose mixed with the feed.

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