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SOME FACTORS INFLUENCING THE BACTERIAL CONTENT AND KEEPING QUALITY OF EGGS.

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SUMMARY.

1. Almost all the eggs containing bacteria were infected in the yolk, while very few of them showed bacteria in the white. very few of the bacteria in eggs grow at blood temperature, while they grow abundantly at room temperature. This is of special interest because of its bearing on the hatching quality of eggs.

2. The number of infected eggs increases slightly with the age of the fowl.

3. Eggs from different hens vary widely in bacterial content and keeping quality. The extremes for the whole period are:

Per cent of eggs infected — minimum 15, maximum 42.
Per cent of eggs spoiled-minimum 4, maximum 34.

4. The eggs from the same hens vary widely in bacterial content and keeping quality at different times, and without apparent cause.

5. When the fowls were given range the number of infected eggs decreased.

6. Feeding wet mash produced an appreciable rise in the number of infected eggs. This increased infection was due to bacteria growing at blood temperature.

7. Mating of the hens did not increase infection of the eggs, as determined by our methods. This suggests that —

8. The greatly increased spoilage of fertile eggs is a direct or indirect consequence of the development of the embryo. Besides giving rise to losses from blood rings, etc., the presence of a dead embryo seems to increase the susceptibility of the eggs to decomposition.

9. We have observed frequent and striking divergencies between the number of eggs infected and the number of eggs spoiled. Increase in infection and decrease in keeping quality do not necessarily run parallel because it is the qualitative rather than the quantitive bacterial content that determines keeping quality.

10. It follows that our quantitative method for determining the infection in eggs does not furnish us a very reliable index to the influence of various factors upon the keeping quality of our eggs.

11. Summer eggs show more bacteria than fall eggs, and as shown in bulletin 180, also more bacteria than spring eggs.

12. The ratio of coagulable to uncoagulable nitrogen did not reveal any appreciable influence, of the factors investigated, upon the keeping quality of the eggs examined. Perhaps this method of determining decomposition in eggs, by means of the $R\frac{C}{U}$ will prove useful in grading frozen and desiccated egg products.

13. No grass eggs were laid by a number of hens whose diet consisted chiefly of alfalfa, nor could any green pigment-forming bacteria be detected in such eggs.

Some Factors Influencing the Bacterial Content and Keeping Quality of Eggs.

By L. D. BUSHNELL and OTTO MAURER.*

PURPOSE OF INVESTIGATIONS.

The purpose of the investigations described here is to aid in the decrease of the enormous losses due to the spoilage of eggs, which, according to the conservative estimate of Hastings, amounts to about 17 per cent of the total value of the crop, or $45,000,000 a year, in the United States. To effect a reduction of these losses we must know what factors and conditions are responsible for the deterioration of the eggs. In this bulletin we are interested primarily in those changes that are of microbial origin. Here the fundamental question arises—Does the destructive infection of eggs occur before or after they have been laid? In other words, is the spoilage due to lack of vitality, digestive disturbances of the fowls, and other factors that facilitate infection during the formation of the egg, or is it due to improper handling of the eggs?

To answer this question we must compare the bacterial contents and keeping quality of fresh eggs produced under various conditions of feeding, etc., with the same criteria in eggs that have been subjected to the various methods of handling customary in the egg trade.

The following considerations will convince the reader that there is ample reason to assume that the bacterial content and keeping quality of eggs may be strongly modified both by factors entering into action before and after the eggs are laid.

GENERAL CONSIDERATIONS.

INFECTION BEFORE THE EGGS ARE LAID.

FACTORS THAT MAY INFLUENCE THE INFECTION OF EGGS.

Let us first consider the possibilities for infection of the eggs before they are laid, and the factors that might be expected to modify it. The vitality of the fowl is of the greatest importance in this respect. All investigators that have studied

* Resigned September, 1913.

the subject (Horowitz, Laschtschenko, Wurtz, Rettger, Sperry) agree that the egg white is strongly bactericidal for such bacteria as *B. anthracis, B. subtilis, Proteus zenkerii, P. zopfii;* while other organisms, such as *Proteus mirabilis, P. vulgaris, B. fluorescens liquefaciens,* and *B. coli,* are much less subject to this action. The bactericidal properties are not due to the presence of a bacteriolytic amboceptor and complement, for complement deteriorates very rapidly at room temperature, while the bactericidal action of egg white lasts for weeks and even months. The addition of yolk to the white inhibits the bactericidal action of the latter; perhaps this is due to an inhibitive action of the fat upon the bactericidal substances. This antilytic action of the yolk has an analogy in the antilytic action of the fats, as demonstrated by Pick and Pribram in the case of blood serum, for instance.

Turro found that a nonlytic mixture of white and yolk acquired marked bactericidal property upon being kept in the incubator for several months. According to him, a solution of the enzymes from the yolk is responsible for this phenomenon. It seems, however, more plausible to attribute this appearance of a bactericidal action to the decomposition products of the fats and lecithin in the yolk, under the influence of the lipase it contains.

However this may be, the bactericidal property of the white of egg represents a defensive property of the egg and oviduct of the greatest importance. Without this defensive agency the oviduct, and therefore the eggs, would be very liable to infection by way of the cloaca. Such an infection would be very likely to greatly decrease the offspring of the fowls by decreasing the hatching quality of the eggs, and by subjecting the young chick to various infections, somewhat like white diarrhea in their epidemiology, though the latter disease is transmitted through the yolk.

This defensive agency of the egg white not only reduces infection during the formation of the egg, but it also reduces subsequent infection through the shell during the hatching, e. g., when the conditions for such an infection are favorable.

It is a law of universal application that a reduction of the vitality and health of an organism decreases and weakens its defensive agencies, thereby increasing susceptibility to infection. We only remind the reader of the predisposing influence of colds for pneumonia, influenza, etc.; also, of the resistance-

lowering effect of malnutrition, fatigue, etc. It seems logical, therefore, to assume that a defensive agency like the bactericidal property of the secretions of the oviduct will not be an exception to this rule. A poorly fed, badly cared for, lousy chicken can not be expected to destroy bacteria, which may find their way from cloaca into the oviduct, as promptly as a vigorous, well-fed bird enjoying the best of health. Moreover, in poorly cared for birds, the condition of the digestive tract is especially conducive to infection of the oviduct on account of such abnormal conditions as diarrhea, constipation, and numerous similar digestive disturbances.

It would be interesting to study the influence of such conditions upon the infection of the oviduct and the eggs. The other extreme, over-feeding, should be avoided just as carefully as under-feeding., In over-fed chickens the abdominal viscera, especially the oviduct, are often so filled with fat that the passage of the egg causes considerable irritation. Antiperistalsis often results in consequence of irritation, as evidenced by the production of such anomalies as double-yolk eggs, eggs within eggs, double-shelled eggs, etc. Also, diseases like the condition designated as egg-bound, which frequently leads to death, may result. Even if the antiperistaltic waves are not strong and frequent enough to produce such striking results, there exists the possibility that such antiperistaltic movements may carry fecal bacteria from the cloaca to the oviduct, resulting in an infection of the eggs, if the bacteria are not promptly destroyed.

Infection by Way of the Yolk. We have thus far considered the influence of vitality of the fowl, digestive disturbances, etc., with regard to the infection of the eggs via the oviduct. Probably of much more importance is the infection through the ovary, for as we have shown in a previous publication (Bulletin 180, of the Kansas State Agricultural Experiment Station), by far the largest number of eggs are infected in the yolk, and only very few in the white. If the defensive agencies of the bird, such as phagocytosis, bacteriolysis, etc., are weakened, bacteria that find their way from the lumen through the intestinal wall into the circulation stand a much better chance to survive and be deposited in the ovary and yolks, where they find conditions favorable for multiplication. Here, again, improper feeding and resulting digestive disturbances may play a very important role. First, these conditions exert a systemic

effect, lowering the general vitality and resisting powers, and facilitating the survival of bacteria that have reached the circulation, thus favoring their deposition in the ovary. Second, these conditions are liable to produce local disturbances in the intestines, amongst others effecting changes in the intestinal wall, that increase its permeability to microorganisms and toxins.

We see these local and systemic disturbances go hand in hand, and cooperate, so to speak, in bringing about an infection of the ovary. This suggests that the diarrheas and other digestive disturbances so common in many poultry yards may become very deleterious to the keeping quality, and, of course, also the hatching quality of the eggs.

RELATION BETWEEN DIET OF CHICKENS, INTESTINAL FLORA AND KEEPING QUALITY OF EGGS.

The diet of a chicken may exert an influence on the keeping quality of the eggs by modifying the bacterial flora of the intestines and, therefore, of the eggs. This influence is quite independent of the increased infection which is likely to follow actual digestive or nutritional disturbances resulting from unbalanced rations, improper or dirty feed.

The diet may modify the intestinal flora in two ways: First, the food, such as milk, cheese, and in the case of chickens especially, dirty food scratched for on the manure heap, may contain large numbers of bacteria which may flood the intestinal tract. Second, the chemical constitution of the food may so modify the nutrient substratum of the intestinal bacteria that certain organisms, originally present in relatively small numbers, find more favorable conditions and grow so abundantly as to change the whole picture of the intestinal flora. Most foods combine these two modifying factors, though in widely different proportions. Let us cite a few examples of the influence of diet upon the intestinal flora, that are of special interest in connection with our subject.

Cantu found *B. proteus* in 67 per cent of the samples of feces from pullets that were fed on meat, while the feces of grain-fed birds showed this organism in only 13 per cent of the samples examined.

Herter and Kendall state the following: "Our experiments in kittens and monkeys show that an abrupt change in diet from a dominantly protein (meat and eggs) to a milk and sugar diet is followed by an alteration of physiological condi-

tions in three distinct ways: *(a)* in the nature of the intestinal flora; *(b)* in the putrefactive products of the feces and urine; *(c)* in the clinical conditions."

The chief characteristic of the bacterial change is the gradual but rapid substitution of an acidophitic, nonproteolyzing type of flora for a strongly proteolyzing type. The chief feature of the putrefactive conditions in the intestine is the reduction of the indol, skatol, phenol, and bound hydrogen sulphide and a diminution in the indican and aromatic oxyacids in the urine. Clinically the most striking feature of the change in diet is an improvement in spirits and activity, which may safely be considered as showing a markedly improved sense of bodily and physical well-being.

Jacobson found that even such a comparatively mild change in diet as from their mother's milk to human milk completely altered the intestinal flora of young dogs. *B. bifidus* appeared in large numbers and replaced the normal bacteria to such a degree that the flora closely resembled that of breast-fed babies. This diet did not, however, agree with the dogs for they rapidly lost weight.

While the difference between a high carbohydrate and a high protein flora is universally recognized and finds serious consideration in dietetic therapy, the modifications of the intestinal flora determined by proteins and different carbohydrates have received very little attention. The future may reveal relations between different proteins and the intestinal flora and its products that may find valuable application in dietetic, therapy.

When we consider, on the one hand, how diet modifies the intestinal flora, and also how, on the other hand, different bacteria vary widely in their ability to bring about a spoilage of the eggs, we may well suspect that diet influences the quality of the eggs. We must always remember that the bacteria in the eggs are derived directly—via oviduct—or indirectly—via yolk—from the intestines. Therefore, any change in the intestinal flora may be accompanied by a change in the flora of the eggs and consequently a change in the keeping quality of the eggs.

INFECTION AFTER THE EGGS ARE LAID.

Improper Handling. Many eggs that are free from bacteria when laid become subsequently infected on account of improper handling. We always have to remember that hac-

teria, under favorable conditions, may penetrate by way of the numerous pores in the shell through the egg membranes and infect the egg content. As long as an egg is perfectly dry this can not occur, but as soon as it is kept in moist or clamp quarters, the bacteria and other microörganisms, which are always abundantly present on the shell, grow and move through the pores and finally reach the egg content to begin their work of destruction. A remuse of the publications dealing with the penetration of bacteria through the eggshell is given in our bulletin 180, of this Experiment Station. It is sufficient, therefore, to state here that Poppe has observed the penetration of many motile organisms into the egg content from fecal material with which the eggs were soiled. Unfortunately, farmers generally take very little care to keep their eggs dry and clean. The nests in which the hens are supposed to lay their eggs, if any are provided at all, are sometimes extremely dirty and get wet in rainy weather. It does not matter if the eggs do get dirty, the farmer thinks, as one does not eat the shell. Dirty nests are a prolific source of loss, because the eggs get soiled with droppings, the bacteria of which get into the egg content. Also, dirty eggs are often washed, and washing reduces the keeping quality of an egg. The egg is covered with a delicate film of dried mucous which serves as a protection against the entrance of bacteria to the interior. Washing removes this film of mucous, thereby favoring infection.

Sudden changes of temperature should also be avoided, because they often lead to sweating of the eggs. Many farmers, as they collect their eggs, wash the dirty ones, or rather dilute the dirt and rub it all over the egg with an old rag. Of course, some of the material is also rubbed into the pores. The wet eggs may then be put into the cellar, or some other cool spot. In summer the rapid change of temperature from the hot pen to the cool cellar causes considerable contraction of the egg contents. Some liquid that is on the shell or in the pores is therefore drawn into the interior. As this liquid is full of bacteria, it will prove very deleterious to the egg content.

Influence of Quality of Shell. Probably the quality of the eggshell has considerable influence on the keeping qualities of the eggs. An irregular, thin shell is probably more favorable to infection than a regular thick one.

A thin shell also cracks much more easily, and, of course, such cracks almost invariablly are followed by infection of the contents. Thin shells, especially if they are cracked, also favor rapid evaporation of water from the white, thereby increasing the air space. Since the size of the air space is usually regarded as an index to the freshness of the egg, a thick, uniform shell is desirable for this reason alone. Moreover, eggs with a good regular shell suffer much less loss through breakage. Breakage not only produces a direct loss, but it induces, indirectly, the loss of other eggs that get soiled and infected with the contents of the broken egg. All these losses can be reduced by liberally supplying the chickens with oyster-shell and by candling all eggs before marketing. All cracked, irregular, and thin-shelled eggs should be used for home consumption. If they are shipped they will be a loss and the cost of their production and handling will have to be borne by the good eggs that survive shipment..

These considerations show that we have ample reason for assuming that the bacterial content and keeping quality of eggs are greatly modified by factors entering into action before, as well as after the eggs are laid. To obtain some information on the infection that takes place during the formation of the eggs, and the relation between such infection and keeping quality, the following experiments were arranged :

PLAN OF EXPERIMENTS.

Two pens, called 20 and 21, respectively, were stocked with thirty-two birds each. The pens were supplied with trap nests, which enabled us to keep individual egg records. The two pens were successively kept under the following conditions :

PEN 20.

March 2 to March 15, male bird in pen.
March 16 to April 19, male bird in pen.
April 20 to May 24, male bird in pen.
May 25 to July 5, without male bird.
July 6 to August 9, without male bird.
August 10 to September 13, without male bird.
September 14 to October 11, without male bird.

PEN 21.

March 2 to March 15, male bird in pen.
March 16 to April 19, without male.
April 20 to May 24, with male.
May 25 to July 5, without male bird until October 11.
July 6 to August 9, fed sour milk.
August 10 to September 13, regular feed.
September 14 to October 11, fed wet mash.

Except for the differences pointed out above, the birds were kept under strictly identical conditions. The effects of feeding sour milk and dry and wet mash were investigated for their influence upon the bacterial content and the keeping quality of the eggs, because they are the ones mostly practiced on poultry farms. Moreover, the far-famed reputation as modifiers of the intestinal flora which various forms of sour milk enjoy especially suggested the investigation of a sour-milk diet. The influence of feeding wet and dry mash, respectively, upon the bacterial content was investigated because they are the methods of feeding most generally practiced, and because there is much discussion as to their respective values.

Description of Fowls and Pens. The birds in each pen consisted of seven four-year-old, seven three-year-old, twelve two-year-old and six one-year-old White Leghorn hens—thirty-two altogether. They were numbered 1 to 32 in pen 20, and 33 to 64 in pen 21.

The pens were of the shed-roof type, with an open front facing south; the floors were 10 x 12 feet, and the pens were 8 feet high in front and 5 feet in the rear. The floor was always covered with a thick litter of straw, in which the birds had to scratch for their grain. The size of the yards surrounding the pens was 100 feet by 150 feet. These yards were kept in grass, and the chickens always had access to them after April 20.

Feeding. Each pen was equipped with one grain can, one dry-mash hopper, one grit hopper, and one hopper for cracked oyster-shell. The whole grain was scattered on the floor in the morning and evening, and the birds had free access to the mash during the entire day. The hens normally received a grain ration consisting of wheat 10, corn 10, oats 10.

The dry mash was composed of wheat bran 4, wheat shorts 6, corn meal 4, meat scraps 5, alfalfa meal 1, oil meal 1.

This was fed in the proportion of twice as much grain as mash, which gave the desired nutritive ratio of 1: 4.6, which is now considered a balanced ration for the laying hen.

The only change in this ration was made when sour milk was fed to pen 21. This was fed in a separate dish, and not mixed with the other feed. While the milk was fed, no meat scrap was added to the mash. The thirty-two hens in pen 21 were fed about one gallon of milk per day, which was about all they could eat. Fed in this quantity the ration was somewhat narrowed, to which change the birds responded by a marked increase in the egg production.

Whenever the birds were kept with a male, two male birds were provided for each pen and they were alternately admitted to the hens, so each one of them could recuperate every other day.

Treatment of Eggs. All the eggs from hens with even numbers were subjected to bacterial analysis, to determine the percentage of infected eggs. In this manner we expected to determine the influence which fertilization and the above-mentioned conditions of feeding exert on the bacterial content of the eggs. The keeping quality does not necessarily run parallel with the amount of bacterial infection of the eggs, as far as the quantitative bacterial count goes. Keeping quality is determined not so much by the number of bacteria present as by their kind. There are many bacteria that produce very little change in the egg, though they may be able to grow and multiply. For this reason, we speak of destructive infection whenever we have in mind that infection which produces the spoilage in the eggs. To determine the influence of the conditions under investigation, upon the keeping quality of the eggs, the following procedure was adopted.

METHOD FOR DETERMINING THE KEEPING QUALITY.

The eggs from all the hens with odd numbers were kept for twelve weeks at a constant room temperature, in an insulated chest that was provided with cold water pipes to lower the temperature during the hot summer months. The eggs were placed in pasteboard containers, turned every week, and candled every month, note being made of all changes. At the end of twelve weeks they were again carefully candled. All eggs that showed decomposition before the candle were subjected to qualitative bacterial analysis (isolation of the destructive

microörganisms), Those eggs that ala not snow any deterioration upon candling were broken into a white porcelain dish and examined carefully, note being taken of evidences of decomposition, such as bad odor, decreased viscosity, marked discoloration, etc. All eggs showing such slight changes are reported as tainted. The sum of all eggs showing evidences of spoilage, either before the candle or upon breaking, are reported as spoiled.

CHEMICAL METHOD FOR DETERMINING KEEPING QUALITY.

To judge the keeping quality of our eggs more accurately, and to find the relation between bacterial content and keeping quality, it was thought desirable to determine the amount of decomposition in the stored eggs more accurately and objectively than our senses permit us to do. Efforts were made, therefore, to work out a simple chemical method for determining the amount of decomposition. We finally adopted the following procedure as the most simple and promising.

The stored eggs that did not show any changes upon candling were broken and thoroughly mixed by means of an egg beater, the eggs laid on three consecutive days being used collectively. The ratio of coagulable to non-coagulable nitrogen was then determined in the mixture. Of course, the eggs from the two pens were broken separately. The ratio of coagulable to non-coagulable nitrogen was obtained as follows.

The eggs are broken and thoroughly mixed with an egg beater. 100 cc. of this egg meat is added to 200 cc. of a 5 per cent sodium chloride solution. This gives a perfectly uniform suspension. With a pipette 10 cc. of it are transferred to a Kjeldahl flask and 100 cc. of isotonic salt solution and 5 cc. of a 1 per cent acetic acid solution are added. The flask is placed in the steam sterilizer and heated for half an hour in streaming steam. The contents are filtered and washed directly into a second Kjeldahl flask. It is important that the washing be done very thoroughly. Before the last two washings the coagulate on the hardened filter paper is broken up (squeezed against the funnel) with a glass rod, so that all the soluble nitrogen contained in it will go into the filtrate. Then the total nitrogen is determined in both flasks by the Kjeldahl method. In our work the Gunning modification was used. Three determinations were made in each sample and the average of the results taken. The ratio of coagulable to uncoagulable nitrogen is obtained

by dividing the number of cubic centimeters of acid neutralized by the distillate from the first flask, with the corresponding value of the second flask. This ratio will, in the following pages, be represented by the symbol $R\frac{C}{U}$.

This method has the advantage that no weighing is required. No matter how much or how little of the egg meat is used, the ratio of the two forms of nitrogen is always the same. As the first stage of the decomposition of the coagulable proteins consists in their degradation to albumoses and peptones which are not coagulated by heat, it was thought that $R\frac{C}{U}$ might serve as an index of bacterial decomposition in the eggs.

Bacteriological Technique. The method used is practically the same as that described in Bulletin 180, with the addition of some extra precautions for preventing contamination from the hands of the operator. We proceeded as follows:

The egg is cleaned with brush and soap and immersed for ten minutes in a 1:500 solution of corrosive sublimate. It is transferred with sterile crucible forceps to a small conical graduate, acute pole uppermost. The corrosive sublimate is removed and the egg dried by washing it first with alcohol and then with ether. The acute pole is scorched to kill spores, etc., that might remain. The egg is then immediately removed from the graduate by the operator's holding it near the blunt pole, turning the acute pole down. The hands of the operator should have been thoroughly greased with vaseline to avoid contamination of the flasks by bacteria that might rub off the hands while handling the eggs. With sharp, stout forceps, which have been sterilized in the flame, a hole about one-half cm. in diameter is made into the acute pole. Holding the egg with the acute pole down, and making the stab from below, prevents contamination from above. The shell around the hole is flamed briskly and the egg is put with the acute pole upon the neck of a tall 300 cc. Erlenmeyer flask containing 100 cc. of sterile bouillon. The blunt end of the egg is now heated with a Bunsen flame, while a close watch is kept on the hole. The heating expands the air in the air space and this expels the contents of the egg. As soon as about half of the albumin has run into the flask the heating is interrupted. The cotton

plug is quickly removed from a second sterile flask, the neck of the flask is flamed, and the egg is transferred from the first to the second flask. Sometimes it is necessary to invert the egg, as soon as the heating is discontinued, to prevent all the albumin from running into the first flask. In this case it often happens that a little of the egg content runs down the outside of the shell, where it may become contaminated. To prevent such material from getting into the next flask, it is cemented to the shell by being heated with the flame. The expulsion of the albumin into the second flask should be done slowly and watched closely. As soon as the yolk appears in the hole the heating is interrupted and the egg is tilted from one side to the other to allow the rest of the albumin to run out. In the same manner the yolk is expelled in two portions. The success of this method depends largely upon the size of the hole. If this is too small, it is hard to separate the white from the yolk; if it is too large, it is difficult to expel the yolk in two separate portions. Sometimes the yolk obstructs the hole before all the albumin is obtained. If the yolk does not retract after cooling, the egg is inverted for a moment. Often the vitelline membrane will not rupture, and the yolk will come out in one piece. This can be prevented by puncturing the membrane with a sterile platinum needle. In this manner four flasks are obtained from each egg, two of them containing albumin and two of them containing yolk. The flasks are repeatedly shaken to mix the contents well. It is of advantage to have tall flasks, because the contents can be mixed more easily without wetting the cotton plug.

Two flasks, one with albumin and one with yolk, are incubated at 38° C. for forty-eight hours, and the other two flasks are incubated at about 20° C. for five days. After this period of development subcultures on agar slants are made to determine if growth has taken place.

An effort was made to identify the organisms obtained in these subcultures, as it was of interest to know whether the bacterial flora of the eggs changed with the diet. Also, the species of bateria present in eggs have recently received considerable attention in connection with the problem of judging egg products, such as frozen and desiccated eggs. A list of the bacteria identified from the eggs will be published later.

TABLE I.

Treatment of Pens and Date of Treatment.	Both pens with males. March 2 to March 15.		Pen 21 without male. March 16 to April 19.		Both pens with males. April 20 to May 24.		Both pens with males. May 25 to July 5.		Pen 21 sour milk. July 6 to August 9.		Both pens normal. August 10 to September 13.		Pen 21 wet mash. September 14 to October 11.	
Number of pen	20	21	20	21	20	21	20	21	20	21	20	21	20	21
Number of eggs analyzed	74	85	295	316	240	264	287	304	244	284	114	163	12	69
Per cent infected *Y*	37.8	32.9	30.1	31.	17	14.7	22.3	22	16.8	13	22.8	18.4	25	30.4
Per cent infected *W*	16.2	14.1	4.1	4.4	.8	3	2.1	2.9	2.4	2.1	1.7	.....	.....	.....
Total per cent infected	40.5	37.6	31.5	33.8	17.8	17	23.3	24.0	18.8	15.1	23.6	18.4	25	30.4
Number stored	.....	.....	310	253	236	191	233	225	239	254	142	118	25	30
Per cent rots	.....	.....	5.1	4.3	4.6	3.6	11.1	6.6	5	3.1	6.3	2.5	4	3.3
Per cent spots	.....	.....	2.2	1.1	7.2	6.3	4.7	3.1	8.3	8.2	2.8	10.1	4	3.3
Per cent tainted	.....	.....	11.6	13	4.2	2.6	1.7	5.3	6.6	7.1	.....	3.4	.....	.....
Per cent spoiled	.....	.....	19	18.5	16.1	12.5	17.6	15.1	20	18.5	9.1	16.1	8.4	6.6
Per cent infected *Y* 38° C	10.8	4.7	3.7	6	1.7	2.6	3.8	3.6	4.5	3.1	7	3.6	8.3	13
Per cent infected *W* 38° C	6.7	5.8	1.3	1.9	.....	.7	.....	.....	.8	1	.....	.....	.....	.....
Per cent infected *W* and *Y* 38° C	2.7	2.3	.....	.....	.....	.....	.....	.....	.....	0.3	.....	.....	.....	.....
Per cent infected *Y* 20° C	35.1	30.5	27.4	28.1	15.4	14	18.4	19.7	13.1	10.5	16.6	15.3	25	21.7
Per cent infected *W* 20° C	13.5	11.7	2.7	2.5	.8	2.2	2.1	2.9	2.4	2.1	1.7	.....	.....	.....
Per cent infected *W* and *Y* 20° C	13.5	7	2.3	.....	.....	.3	1	.9	.4	.8	.8	.....	.....	.....

RESULTS.

Table I shows the influence of different methods of feeding, etc., on infection and keeping quality, and also the seasonal variation in the bacterial content and keeping quality of the eggs.

DISCUSSION OF RESULTS.

BACTERIA IN EGGS, GROWING AT DIFFERENT TEMPERATURES. THEIR DISTRIBUTION IN WHITE AND YOLK.

The tables show, thereby confirming our previous work, that the number of eggs showing bacteria that grow at room temperature is very much larger than those showing growth at blood temperature. No doubt this difference is of great importance in connection with the hatching quality of the eggs. Since most of the bacteria in nornlal eggs can not develop at blood temperature, they can not interfere with the growth of the embryo. This does not mean, however, that the bacteria which grow at room temperature have no influence on the hatching quality of the eggs. They may develop during the time intervening between the laying of the eggs and their incubation. It is easily possible that even a very small amount of decomposition produced in this manner may prove very deleterious to the delicate embryo chick.

The difference between the bacterial content of white and yolk is also in agreement with our former results. It would be hasty, though, to conclude from this difference that infection of the eggs usually takes place in the ovary. The bacteria, especially in the upper part of the oviduct where they are very close to the yolk, may survive the bactericidal action of the white for a sufficiently long time to permit them to invade the yolk—chemotactic substances diffusing from the yolk in traces, may play a role in such an invasion.

Once in the yolk the bacteria are safe, for the yolk does not possess bactericidal properties.

The following table representing a summary for both pens and for the whole of the experiment, was compiled to show at a glance the differences pointed out in this paragraph.

TABLE II.

Total number of eggs analyzed.....	2,759
Per cent of eggs infected	23.7
Per cent of eggs infected in yolk, 38° C.....	4.3
Per cent of eggs infected in white, 38° C. ...	0.8
Per cent of eggs infected in yolk, 20° C.	19.3
Per cent of eggs infected in white, 20° C.....	2.6

INFLUENCE OF AGE OF HENS UPON INFECTION OF EGGS AND KEEPING QUALITY.

The following table has been arranged to show the relation existing between the age of fowls, infection, and keeping quality of eggs.

TABLE III.—AVERAGE OF BOTH PENS.

	1 year old.	2 years old.	3 years old.	4 years old.
Number eggs analyzed.....	443	1,152	592	549
Number infected	98	265	149	147
Per cent	22	23	25	27
Number stored	473	900	455	402
Number spoiled	58	169	96	49
Per cent spoiled.....	12	18.7	21	12

Comparing the average infection of both pens, for the different ages, we find that there exists a slight tendency for the infection to increase with the age of the birds. The number of infected eggs increases about 2 per cent from year to year. As regards the keeping quality, it seems surprising that the eggs from the two- and three-year old birds show about 8 per cent more infection than the other ages. This incongruity between the number of infected eggs and the keeping quality will often be met with in the course of the discussion of our results.

TABLE IV.—INDIVIDUAL EGG RECORD,

No. hen.	Number analyzed.	Per cent infected.	Number stored.	Per cent spoiled.
1.....	...	....	88	13.6
2.....	36	15.1	...	....
3.....	...	....	117	11.9
4.....	79	27.9	...	....
5.....	...	....	65	15.8
6.....	40	27.5	...	....
7.....	...	....	35	20.0
8.....	108	28.7	...	....
9.....	...	....	52	28.8
10.....	87	22.2	...	....
11.....	...	....	99	11.1
12.....	54	24.1	...	....
13.....	...	....	40	1.5
14.....	111	20.7	...	....
15.....	...	....	88	11.3
16.....	106	26.4	...	....
17.....	...	....	72	26.3
18.....	59	20.3	...	....
19.....	...	....	25	4.0
20.....	101	21.7	...	....
21.....	...	....	59	33.8
22.....	99	27.2	...	....
23.....	...	....	101	15.8
24.....	109	18.3	...	....
25.....	...	....	100	30.0

TABLE IV—CONCLUDED.

No. hen.	Number analyzed.	Per cent infected.	Number stored.	Per cent spoiled.
26	27	18.5	...	....
27	...	....	90	8.8
28	89	16.8	...	....
29	...	....	61	32.7
30	59	42.3	...	....
31	...	....	72	4.1
32	16	31.2	...	....
33	...	....	60	13.3
34	88	23.8	...	....
35	...	....	48	14.5
36	90	20.0	...	....
37	...	....	105	7.6
38	56	28.5	...	....
39	...	....	64	25.0
40	112	....	...	....
41	...	....	13	27.4
42	86	26.7	...	....
43	...	....	64	23.4
44	114	15.7	...	....
45	...	....	60	16.6
46	71	21.1	...	....
47	...	....	49	20.4
48	146	21.2	...	....
49	...	....	116	9.4
50	83	26.5	...	....
51	...	....	78	26.9
52	97	40.2	...	....
53	...	....	11	9.1
54	97	18.5	...	....
55	...	....	71	21.1
56	75	17.3	...	....
57	...	....	88	13.6
58	112	24.1	...	....
59	...	....	61	8.2
60	10	30.0	...	....
61	...	....	47	14.9
62	64	23.4	...	....
63	...	....	58	6.9
64	100	25.0	...	....

This table was made up to see whether infection is limited to certain hens, and whether certain hens have a special tendency to lay infected eggs, and eggs that are likely to spoil. If all the infected and spoiled eggs were derived from a few hens only, that constantly lay infected eggs, then we would have to assume that infection of the eggs is due to a pathological process of the oviduct, or more likely the ovary, as, *e. g.*, in white diarrhea.

Our table shows no hens that did not lay infected eggs during this long period of investigation. The minimum is 15.1 per cent infected out of a total of 86 eggs analyzed from hen 2, while the maximum of 42.3 per cent infected out of 59

was shown by hen 30. In further looking up our records we often find a very large percentage of infected eggs during certain periods. For instance, 40.2 per cent of all the eggs laid by hen 52 were infected. The same hen laid 20 eggs between March 10 and April 19, 60 per cent of which were infected. An illustration to show how widely the bacterial content of eggs from the same hen varies at different times is given by hens 34 and 36.

TABLE V.

HEN 34.

Date.	Number analyzed.	Number infected.	Per cent infected.
March 15 to April 19	20	9	45.0
April 20 to May 24	13	4	30.7
May 25 to June 28	18	1	5.5
June 29 to August 2	20	1	5.0
August 3 to September 16	2	0	0.0
September 16 to October 11	14	6	42.8
Total	88	21	23.8

HEN 36.

March 15 to April 19	15	7	46.6
April 20 to May 24	18	3	16.6
May 25 to June 28	6	1	16.6
June 28 to August 2	19	0	0.0
August 2 to September 16	12	1	8.3
September 16 to October 11	16	5	31.2
Total	90	18	20.0

The average percentage of infected and spoiled eggs for the whole experiment is 23.7 per cent infected and 16.1 per cent spoiled. The spoiled eggs again were made up of rots 4.9 per cent, spots 5.1 per cent, and tainted 6.1 per cent.

The eggs obtained from different hens also vary widely in their keeping quality. For instance, of the eggs laid by hens 19 and 31 only 4 per cent spoiled, while 33.8 per cent of the eggs laid by hen 21 spoiled. This is a difference of almost 30 per cent. Unfortunately, we have, at the present time, no means of eliminating hens the eggs of which show an exceptional susceptibility to spoilage.

A more detailed study of our individual egg records shows us the same phenomenon with regard to the spoilage of the eggs, as was pointed out above with regard to infection. The number of spoiled eggs from the same hen varies widely and irregularly at different times, even under identical conditions of surroundings, feed, etc. No law has been traced, so far, that governs these variations.

Influence of Range on Bacterial Content of Eggs. In looking over our summary we find a marked and sudden decrease of the number of eggs infected after April 19, a decrease which is equally marked in both pens and which, with minor variations, lasts until September or October. In looking for the cause of this peculiar phenomenon we find that on April 20, the birds in both pens were given the use of the yards surrounding the pen, while up to that date the birds had been shut up in the pens all the time, to give the vegetation in the yards a good start. This peculiar result is probably due to the increased well-being of the birds and to the change in their diet (green food, insects), resulting from range.

Seasonal variations might be looked for as another explanation for this change. However, the following considerations will show this to be untenable. In bulletin 180 we gave the following data:

Per cent of eggs infected, pen 20, April 20 to May 4, 16.5 per cent; May 4 to June 4, 26.8 per cent.

These results show a higher infection for the summer eggs than for the spring eggs. Lamson, too, states that summer eggs contain more bacteria than spring eggs. It appears justifiable, therefore, to conclude that the decrease of the number of eggs infected after April 20 was not due to seasonal variations, but to the range which the birds are given.

INFLUENCE OF THE CONDITIONS UNDER INVESTIGATION, UPON THE BACTERIAL CONTENT AND THE KEEPING QUALITY OF THE EGG.

Wet and Dry Mash. Let us consider the most striking results revealed by our summaries. First, from August 10 to September 13, when the two pens were kept under identical conditions, the percentage of infected eggs is about 24 in pen 20 and 18 in pen 21. From September 14 to October 11, when pen 21 received a wet mash, the data are 25 per cent for pen 20 and 30 per cent for pen 21. The increase in infection is therefore 25—24=1 per cent for pen 20, while it is 30—18=12 per cent for pen 21. This large increase in infection in pen 21 as compared with pen 20 can hardly be attributed to experimental errors alone. Let us see what this error is: the difference between the two experimental pens when kept under identical conditions was never more than 5 per cent. This was from August 10 to September 13. Pen 21, therefore, shows an increase of 6 per cent above the experi-

mental variation as compared with pen 20. This difference should be attributed to the feeding of the wet mash. By looking at the last four columns of Table I we find that this increase in infection in pen 21 is due entirely to an increase of bacteria that grow at blood temperature. It would be interesting to make further studies, therefore, on the influence of feeding wet mash upon the hatching qualities of the eggs, for it seems quite likely that the bacteria which grow at blood temperature interfere with the normal development of the embryo.

When we consider the relation between infection and keeping quality, as influenced by wet mash, we obtain very startling results, The per cent of spoiled eggs for pen 20 is 1 per cent lower during the period from September 14 to October 11 than during the previous period from August 10 to September 13. Thus we have the apparently incongruous result that both keeping quality and bacterial content increased at the same time. This is possible, of course, for the reasons discussed on a previous page, namely, that keeping quality depends not only upon the quantity of infection, but primarily upon the quality. We have reason to doubt, however, whether this explains the above divergencies between infection and spoilage. A glance at Table I shows that the two pens vary widely in the number of spoiled eggs during the period from August 10 to September 13, these numbers being 9.1 per cent for pen 20, and 16.1 per cent for pen 21. Since these two pens show only a comparatively slight difference in their eggs during the two preceding periods, we are unable to account for this wide divergence during the period of August 10 to September 13.

Fertilization. A perusal of Table I does not reveal any influence of fertilization upon the bacterial content or keeping quality (at 20-25° C.) of our eggs. The table shows differences, of course, but these are neither constant nor large enough to permit of any positive conclusion. If fertilization did increase the bacterial infection of eggs, then we would expect such an increase to be especially striking in the white of the eggs, because the nearest explanation for such an infection would be a mechanical transport of bacteria into the oviduct during copulation and fertilization. No such increase of infection in the white could be detected.

The fact that our experiment did not show any influence of fertilization upon bacterial content and keeping quality of the eggs under our experimental conditions suggests that the enormous losses invariably experienced with fertile market eggs is due primarily to development of the embryo, and not to an increased bacterial content of such eggs. Far be it from us, on the basis of these results, to underestimate the tremendous losses due to the fertilization of market eggs. Incubation of such eggs, without increased bacterial content, is sufficient to give rise to tremendous losses. The economic importance of the subject justifies us in dilating a little on the different ways in which the incubation of fertile eggs gives rise, to losses. Lamon and Opperman state that it takes 24 hours for the embryo to devolop sufficiently to be recognized in, candling. At the expiration of 36 hours the presence of blood was easily detected. At a somewhat higher temperature than that normal for hatching, development of the embryo proceeds even more rapidly. At lower temperature development proceeds much more slowly, but even at 90 degrees F. a blood ring can be detected after about eight days. It is easily understood therefore, that during the hot summer months, when, in Kansas for instance, the temperature often rises above 100 degrees F. every day for weeks at a time, it is almost impossible to prevent the deterioration of fertile.eggs.

The losses due to the fertilization of market eggs are far more extensive and complex than is generally supposed. Not only are such fertile eggs liable to be lost on account of incubation (blood rings), but indirectly they often are responsible for the deterioration of originally sound eggs. This is brought about in the following manner: During its develop ment, the embryo derives some substances from the shell as demonstrated by F. Tangle and Hammerschlag. As a result, the shell becomes depleted of both mineral and organic matter, and in consequence becomes very brittle and liable to breakage. Entirely sound eggs may be covered or soiled with the contents. of such broken incubated eggs, furnishing an excellent medium for bacteria to grow and infect the soiled eggs, That incubation often proceeds very far under customary trade conditions is shown by the fact that the egg boxes arriving at the packers not infrequently contain live chicks. This has repeatedly been observed in Topeka and other places in the West where weather conditions during summer are very favorable

to incubation. Even if no invasion of bacteria occurs, the mere soiling of the eggs will materially decrease their market value.

Poppe found bacteria more frequently in the oviduct of mated than of unmated hens, This does not necessarily mean that these bacteria will reach the eggs. As the oviduct begins secreting, under the stimulation of the descending egg, these bacteria might be killed by the bactericidal substances of the secretions. The lymphoid structure of the oviduct also favors phagocytosis and removal of bacteria.

Lamon found that infertile eggs always kept better than fertile ones, even in places where incubation could not take place. Our results indicate that the bacterial content of the eggs is not influenced by mating the hens. We must conclude, therefore, that fertilization makes the eggs more susceptible to bacterial attack, Sooner or later in their journey to the consumer, most fertile market eggs contain a dead embryo. Perhaps this dead embryo forms an especially good culture-medium for the bacteria.

Sour Milk. Our summaries in Table I do not show any effect of sour-milk feeding, either upon bacterial content or keeping quality of the eggs.

RELATION BETWEEN BACTERIAL CONTENT AND KEEPING QUALITY.

We have had occasion, several times in the course of these discussions, to point out that the keeping quality is not necessarily inversely proportional to the amount of infection of the eggs. There are many examples where the number of spoiled eggs decreases as the number of infected eggs increases. Here are the most striking data showing the inconstancy of the relations between quantitative bacterial content and keeping quality.

We see that in some cases, as, for instance, during the fifth period, the number of spoiled eggs comes very close to the number of infected eggs, while in other cases, during the last period for instance, the number of spoiled eggs is only one-fourth or one-fifth the number of infected eggs.

The divergencies between the number of eggs infected and the number of eggs spoiled must not lead us to underestimate the significance of bacterial infection for the spoilage, While not all eggs that contain bacteria will spoil, it is only those eggs that do contain bacteria or other microorganisms that can

TABLE VI.

Period.	March 16 to April 19.		April 20 to May 24.		May 25 to July 15.		July 16 to August 9.		August 10 to September 13.		September 14 to October 11.	
Number of pen	20	21	20	21	20	21	20	21	20	21	20	21
Per cent infected	31.5	33.8	17.8	17	23.3	24	18.8	15.1	23.6	18.4	25	30.4
Per cent spoiled	19.0	18.5	16.1	12.5	17.6	15.1	20	18.5	9.1	16.1	8.4	6.6

spoil to any considerable extent (not considering fertilized eggs showing blood rings, etc.). The decay produced by the enzymes in the white and yolk is practically negligible, except perhaps during cold storage.

Seasonable Variation in Keeping Quality. The plan of these experiments is not favorable for a study of the seasonal variations in keeping quality. For such a study one of the pens should have been kept under the same conditions throughout the experiment. Since pen 20 was kept under the same conditions only after May 25, until the close of the experiment, our study of seasonal variations can cover only the period from May 25 to October 11. The percentages of spoiled eggs are shown in the following table:

TABLE VII.

Date.	Per cent spoiled eggs.	Per cent. infected eggs.
May 25 to July 5.....	17.6	23.3
July 6 to August 9.....	20.0	18.8
August 10 to September 13.....	9.1	23.6
September 13 to October 11.....	8.4	25.0

We see that the spoilage reaches its maximum in July and August and then declines rapidly. The number of infected eggs does not show much variation during the same period—another example of discrepancy between infection and keeping quality.

CHEMICAL RESULTS.

SUMMARY OF NITROGEN RATIOS.

$R\frac{C}{U}$ = ratio coagulable to uncoagulable nitrogen.

TABLE VIII.

PEN 20.

Date.	Treatment.	$R\frac{C}{U}$
Mar. 2 to Mar. 15.....	Male bird in pen.....	Not determined
Mar. 15 to Apr. 19....	Male bird in pen.....	8.68
Apr. 20 to May 24....	Male bird in pen.....	9.21
May 25 to July 5.....	Without male bird.....	8.71
July 6 to Aug. 9.....	Without male bird.....	9.48
Aug. 10 to Sept. 13....	Normal, dry mash, without male bird....	9.42
Sept. 14 to Oct. 11.....	Normal, dry mash, without male bird....	9.75

PEN 21.

Mar. 2 to Mar. 15.....	Male bird in pen.....	7.95
Mar. 16 to Apr. 19....	Without male bird.....	7.95
Apr. 20 to May 24....	Male bird in pen.....	9.18
May 25 to July 5.....	Without male bird.....	9.16
July 6 to Aug. 9.....	Curd milk, etc.....	10.12
Aug. 10 to Sept. 13....	Normal	10.13
Sept. 14 to Oct. 11.....	Wet mash	10.13

When decomposition occurs the uncoagulable nitrogen increases, the denominator U of our fraction $\frac{C}{U}$ increases, so that the ratio decreases. In other words, *e. g.*, R. $\frac{C}{U}$ 2 indicates more decomposition than R $\frac{C}{U}$ 3, or R $\frac{C}{U}$ is inversely proportional to the amount of decomposition.

The results summarized above do not indicate any marked influence of the conditions investigated upon the keeping quality of the eggs analyzed. Generally the ratio for pen 20 is from .4 to .7 higher than for pen 21, even under normal conditions. The period from March 16 to April 19 is an exception to this rule, this condition being reversed. During the period from March 16 to April 19—when the fowls are shut up—the average of both pens is lower than throughout the rest of the experiment. Perhaps this is an expression of the increased infection during this period, which was discussed before.

The bacteria in the eggs, while not able to produce changes that betrayed themselves to our senses, might have produced enough decomposition to be detected by these chemical means.

Perhaps this method for determining the amount of decomposition may find useful application in grading such products as frozen desiccated eggs.

GRASS EGGS.

Eggs in which the albumen shows a greenish discoloration are frequently found in the markets. Many egg dealers believe there exists a casual relation between the feeding of green feed and this greenish discoloration. It is especially alfalfa which is blamed for the occurrence of such grass eggs, or alfalfa eggs, as they are called. The green color is due not to chlorophyll, of course, but to bacterial pigment produced by such organisms as *B. pyocyancus, B. fluorescens liquefaciens*, etc. As all the dealers and farmers with whom we discussed the matter strongly believe in the relation between green feed and grass eggs, we thought that possibly alfalfa had a special pigment-producing flora of its own. Such bacteria, it was thought, might be introduced with the alfalfa in very large numbers, reach the egg, and produce a greenish

discoloration. That an introduction of bacteria in this manner is possible is proved by the observations of Rabner, who observed *B. mesentericus* in chicken feces after, and only after, feeding cabbage.

To determine whether the feeding of alfalfa produces grass eggs, six Barred Rock hens were kept in an alfalfa field. The hens were numbered A, B, C, D, E, and F. All the eggs from B, D, and F were subjected to qualitative bacterial analysis. Although infection was frequent, no pigment-producing organisms could be isolated. Alternate eggs from hens A, C, and E were kept for four weeks at room temperature, and at blood temperature, respectively. They were then broken into a white porcelain dish and carefully examined for greenish discoloration. No grass eggs, however, were found.

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Since completing this manuscript, Bulletin No. 75, "The Bacteriology of the Hen's Egg, with Special Reference to its Freedom from Microbic Invasion," from the Storrs Agricultural Experiment Station, Storrs, Conn., has come to my notice.

In this publication Dr. Rettger gives data showing a much smaller percentage of infection than has previously been published. He considers that the number of eggs actually showing bacteria is probably less than three or four per cent, and that higher percentages undoubtedly show extraneous contamination.

After two years' work in this laboratory we are unable to show results as low as this on any considerable number of eggs. The results given in this bulletin are even higher than those given by Maurer in 1911. Extra precautions were also taken to prevent contamination, as previously noted. Plates exposed in this laboratory showed very few colonies, about ten in fifteen minutes. This means that but one organism would fall upon a plate every 1.5 minutes. Comparing the area of the opening in the top of an Erlenmeyer flask and that of a plate, we find a ratio of about 1: 7. The chance of each receiving an organism in the same length of time is 1: 7. One organism would fall into the top of a flask every 10.5 minutes. As the flasks were open for a very short time, perhaps half a minute, this would allow but one in every twenty flasks to be contaminated, or only five per cent of contamination from the air.

The eggs underwent a rather severe treatment before being analyzed. Treated with 1-500 bichloride of mercury for ten minutes, washed in alcohol and ether, and the pole, where the opening was made, scorched until slightly brown in the flame. I do not think that any but spore forms could withstand this treatment.

A possible source of contamination is the bouillon used. This was placed in flasks and heated at twenty pounds pressure for twenty or thirty minutes in the autoclay. This must kill all vegetable forms.

Of the 571 bacterial cultures isolated, 87, or 15.1 per cent, showed spores. Then less than one-sixth of all the organisms present are likely to be present because of contamination by spores. One-sixth of 23.6 per cent, total eggs infected, is about four per cent. This may be considered as due to spore contamination. This percentage plus the five per cent from the air, these were undoubtedly not all non-spore formers, gives a possible contamination of nine per cent, leaving 14 per cent as against three or four as given by Rettger. This is close to the number that actually spoiled in storage.

In considering the spoilage on storing we find 16.6 per cent spoiled. These eggs were handled with more than ordinary care as to temperature, moisture and external influences, and most of them must have been infected when fresh. While we find a higher percentage of infected than spoiled eggs, this is to be expected, as certain organisms probably remain dormant in the egg and will not grow until placed under more favorable conditions. On the other hand, it is to be expected that certain organisms may develop in the egg, but would not develop under conditions of culture due to improper food, aeration, osmotic conditions, etc.

I am unable to account for the wide discrepancy in results as due entirely to contamination during the manipulation of our cultures.