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Studies on the Effect of the Alteration in the Internal Environment of Poultry Egg on Embryonic Growth

II. A Comparison of Embryonic Growth in Widely Different Chickens in Egg Size*

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Summary

Embryonic growth was studied in relation to egg weight in the following breeds or crossbreds: White Leghorn (WL) and its two kinds of hybrids, embryos from (White Bantam cocks × White Leghorn hens) hens backcrossed to Bantam cocks (BL) and F_2 chicks produced by mating mutually F_1 hybrids of White Leghorn hens with a Red jungle fowl cock (JL); and three Japanese native breeds, a game Bantams (SS), a long-crower (LC) and a White Bantam (WB). The eggs used were characterized by a variation of wider range: the highest ranking eggs weighed 54.85 g in White Leghorn and the lowest ones 29.21 g in White Bantam. Embryos and chicks were individually weighed at 6 critical points from 6 day's incubation to hatching time, to analyse whether any significant differences in embryo weights was dependent on egg size. The newly hatched chicks were weighed within 24 hours after hatching.

1. The differences in embryo weight among the four kinds compared reached high significances at 13 days of incubation although its rank order was not according to egg size. As a rule, the remarkable differences in embryo weights by egg size appeared after 16 days of incubation. A rank correlation (r_s) of egg weight with chick weight showed 0.943 ($P<0.05$).

2. An analyses of embryo or chick weight as a percentage of egg weight indicated that the less the egg weight was, the more the per cent weight declined from 6 to 13 days of incubation. But at hatching time the per cent weight did not correspond to the egg weight order. At hatching time the per cent weight varied considerably in a wide range from 64.3 per cent for BL to 70.7 per cent for SS.

3. The effect of the initial egg weight on the variance of embryo weight was estimated in the coefficient of determination. Although widely fluctuant, the coefficient on the whole showed a positive increase with progressive incubation time.

4. Unassimilated yolk weight at 16 and 19 days of incubation showed that the smaller the egg weight was, the smaller the yoke was. Unassimilated yolk weight as a percentage of embryo (plus unassimilated yolk weight) fluctuated unrelated to the egg weight group.

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For the past five decades, studies on the causes of variation in chick embryo weight have been made by many poultry investigators, some of which were interested especially in the effect of egg weight on growth patterns in chickens. Data concerning the effect of body weight- or egg weight-selection on embryo size have been obtained since 1950: Wiley (1) did not find consistently significant differences between weights of embryos from two strains of Barred Plymouth Rocks selected for large and small egg size, while Coleman et al. (2) in White Rock lines subjected to two-way selection for eight week body weight observed highly significant line differences in wet embryo weights from the fourteenth to the nineteenth day of incubation.

McNary, Bell and Moore (3) studied the growth of inbred and hybrid embryos, reporting that embryos from the heavy lines were consistently larger than those from the Leghorn lines although genetic differences were most easily detected at two weeks.

Reports on the effect of egg weight on growth patterns in the domestic fowl were reviewed historically by Bray and Iton (4) studying embryonic and early chick growth relative to egg weight in five strains of domestic fowl, and they asserted in their own experimentation that egg weight could be regarded as a temporary environmental influence on the differences among strains for embryo weights, chick weights when calculated as a percentage of egg weight.

While it is clear that genetic differences exist in embryonic growth patterns, it would appear in general that embryonic growth is influenced considerably by egg size and by other environmental factors.

In the previous work (5), however, it was thought that the breed or crossbred differences in the weight ratio of albumen to yolk were caused mainly by egg size. The original purpose of the present investigation was, then, to determine the effect of egg weight on the embryonic growth in widely different egg-sized chicken breeds or crossbreds.

Experimental Procedure

Four breed- and two hybrid-embryos of chickens were utilised in this study.

A White Leghorn (WL) and Two Kinds of Hybrid: eight White Leghorn hens at 16 months of age were inseminated with only a Leghorn cock of 10 months of age. Four 16 month old hybrid hens of White Leghorn females with White Bantam (a Japanese Bantam) males were artifiical-inseminated with two White Bantam-pooled semen (one is 34 months old and the other 18 months old) (BL). The insemination was practised every four days. Two kinds of above-mentioned chickens were reared at the Laboratory of Animal Breeding, Tohoku University, and their hatching eggs were layed between the 7th of March and 6th of April, 1965, collected every five days so as to obtain embryos of a given developmental stage. Additional eggs were collected between 18th of April and 25th of May to guarantee an embryo sample size. Some of these hens had been utilised in the previous work (5).

F_2 progenies (JL) were produced by mating mutually F_1 hybrids of White Leghorn hens with a Red jungle fowl cock. These eggs, from which F_2 chicks hatched, layed by four F_1 hybrid hens were introduced from the Laboratory of Animal Breeding, Tokyo University of Agriculture.

Three Japanese Native Breeds: hens laying the relatively smaller eggs sired by unknown cocks involved in two Small Shamos (a Japanese Game Bantam; SS), seven Totenkos (a Japanese long-crower; LC) and seven White Chabos (a Japanese White Bantam; WB). Their eggs were kindly provided for study by a few bird fanciers in Sendai, grouped together, along with eggs from stocks at our laboratory on account of the difficulty in obtaining eggs. Our Laboratory stock data out of these chickens can be described as below: a Shamo cock and a hen at 15 to 26 months of age, a Totenko cock and a hen at 9 months of age, and the same Bantam cock as utilised in BL and three hens at 18 to 34 months of age.

Thus, it was not possible to put these materials in order. All eggs from JL and three native breeds were laid during April in 1965.

The individual eggs were weighed to the nearest one-twentieth of a gram. The design for the embryonic growth study consisted of obtaining wet embryo weight at 6, 10, 13, 16 and 19 days of incubation and newly hatched chick weights. Such being the case previously stated both LC and WB embryos were lacking in 10 and 16 days of incubation, JL and SS being available for chick weights only.

The eggs from each kind were so distributed that more than five embryos might be guaranteed per incubation period even in the most poorly represented sample size.

Wet embryo weights were obtained after the embryos were removed from the eggs and rolled on a moistened filter paper to remove excess moisture and chick weights within 24 hours after hatched. Six and 10 day embryos were weighed to the nearest 0.1 milligram, and the older than at 13 days of incubation and chicks weighed with the same balances as egg weights did. The embryos or chicks were not sexed in this investigation.

Table 1. shows the egg size data with standard errors. Three larger eggs were layed by White Leghorns or their two crosses and the smaller by three native varieties. In contrast with the egg size range of 54.6 to 60.3 grams in Bray and Iton (4), the present materials were characterized by the wider variation from 54.85 grams in WL to 29.21 grams in WB. In addition mean values from each kind would appear inherent in the breeds or crossbreds at present.

The significance of the differences in embryo weights between kinds were analysed by analysis of variance. The mean embryo or chick weight as a percentage of egg weight were analysed by the same procedure which was used to analyse the mean embryo or chick weights. In this case a transformation of percentage data was warranted.

TABLE 1. *Mean Egg Weight (Grams) of Six Breeds or Crossbreds*

WL[a]	JL	BL	SS	LC	WB
54.85 ±0.29[b]	45.29 ±0.84	41.15 ±0.32	40.00 ±0.64	36.65 ±0.46	29.21 ±0.56

a) See text for abbreviations.
b) Standard error of the mean.

Results and Discussion

Embryo or Chick Weight: at 6 days of incubation the differences between the four kinds compared were not significant. Thus, the two kinds behaved in the same way as at the earlier stage, that is, at 10 day's incubation. The differences between them reached high significances at 13 days of incubation although its rank order did not followed the egg size order (Tables 2 and 3). Extremes WL and WB embryos or chicks were significantly larger or smaller than those from any other kinds at 13 days of incubation to hatching time.

TABLE 2. *Mean Embryo or Chick Weight (Grams)*

Age	WL	JL	BL	SS	LC	WB
Embryo 6 days	0.3638 ±0.0128[a]		0.3777 ±0.0177		0.4369 ±0.0203	0.3684 ±0.0111
10	2.59 ±0.05		2.43 ±0.08			
13	8.01 ±0.12		6.71 ±0.13		7.49 ±0.24	6.01 ±0.30
16	16.22 ±0.18		12.29 ±0.36			
19	28.92 ±0.52		20.40 ±0.55		19.23 ±0.56	14.16 ±0.47
Hatched	38.82 ±0.58	30.34 ±0.52	27.20 ±0.76	28.27 ±0.63	26.13 ±0.57	20.02 ±0.90

a) Standard error of the mean.

BL egg size laid by F_1 hybrids from WB males with WL females were intermediate between the extremes. These three embryos were ranked by egg weight and the differences among them were of significance after 13 days of incubation. This fact indicates that embryonic growth depends greatly on egg size through the later incubation periods even though crossing with WL carries a genetic improvement for embryo size. This situation could as well explain the relationship between WL and the two crossbreds, JL and BL at hatching, when these day old chicks in weight were highly different each other. Recent reports (6, 7) indicate that about 30 grams in weight were recorded for eggs layed by Indian, Philippine and Thailand Red jungle fowls introduced to Japan. The mean egg weight from F_1 hybrids of the jungle fowl males with Leghorn females were

TABLE 3. *Ranking and Statistical Analyses of Breeds or Crossbreds by Embryo or Chick Weight.*

Age	Rank by embryo or chick weight Large ← 1	2	3	4	→ Small 5	6
Embryo 6 days	LC	BL	WB	WL		
10	WL	BL				
13	WL	LC	BL	WB		
16	WL	BL				
19	WL	BL	LC	WB		
Hatched	WL	JL	SS	BL	LC	WB

Values not underscored by the same line are significantly different at the level of probability indicated as follows.
Age embryo 13 days: WL-LC at 5% level and any others at 1% level.
Age embryo 16 days: between-two kinds at 0.5% level.
Age embryo 19 days: all differences at 1% level.
Hatched: JL-BL at 5% level and any others at 1% level.

45.21 grams as in Table 1. which as expected was almost intermediate between the parental mean values. The result did show that the hatching weight is due to an improvement for egg size as an embryonic environment, rather than the original breed cock take part in embryo weight. A phylogenetic relation of domestic chickens to the original breed in embryonic growth were ignored in the present experiment.

Hutt (8) determined whether or not the gene *dw* affects the growth of the embryo and chick during the three weeks of incubation, in which chicks were all hatched from hens not dwarfed that laid eggs of normal size, about 57 to 60 grams, and such eggs could impose no limitation on the initial size of the chick, as would the smaller eggs laid by dwarf hens, that is, the gene *dw* does not retard the growth of the chick during incubation. The relationship of egg weight to embryo or chick weight within each kind would be different in the present study as will be seen later. However, it may be explained better, by a maternal limitation to egg weight that imposed on embryo weight, even though the crossbred males in question had sex-linked effects on embryonic growth.

The 19 day embryo weight was ranked identified with the egg weight rank in the four kinds compared at four critical points. This rank correlation in Bray and Iton (4) were consistently zero or negative from 6 to 15 days showed a declining negative relationship from 7 to 12 days, and an increasing positive value from 16 days to hatched weight when the ranking by chick weight corresponded exactly to the ranking by egg weight. A determination of the ranking with respect to incubation period must be carried out in a future investigation, since only the two kinds were known for weight at 16 days of incubation in this study. Although the hatched chicks were identical somewhat incompletely with egg weight ranking ($r_s=0.943$; $P<0.05$), it may be thought the rank correlation as one in nature because of no significant difference between SS and BL at hatching time.

Mean Embryo or Chick Weight as a Percentage of Egg Weight: the less the egg weight was, the more the coefficient inclined from 6 to 13 days (Table 4). At these developmental stages Leghorn embryos were consistently significantly smaller than the other one to three kinds investigated, while Bantam embryos were

TABLE 4. *Mean Embryo or Chick Weight as a Percentage of Egg Weight*

Age	WL	JL	BL	SS	LC	WB
Embryo 6 days	0.67 ±0.03[a)]		0.92 ±0.04		1.23 ±0.04	1.30 ±0.05
10	4.85 ±0.09		6.21 ±0.17			
13	14.4 ±0.2		16.3 ±0.3		20.3 ±1.4	20.6 ±1.3
16	29.8 ±0.4		30.0 ±0.8			
19	51.8 ±0.5		49.0 ±0.9		54.0 ±1.3	49.5 ±2.5
Hatched	70.1 ±0.4	67.0 ±0.7	64.3 ±0.9	70.7 ±1.2	67.9 ±1.3	66.9 ±0.9

a) Standard error of the mean.

TABLE 5. *Ranking and Statistical Analyses of Breeds or Crossbreds by Embryo or Chick Weight as a Percentage of Egg Weight*

Age	Rank by per cent embryo or chick weight Large ← 1	2	3	4	5	→ Small 6
Embryo 6 days	WB	LC	BL	WL		
10	BL	WL				
13	WB	LC	BL	WL		
16	BL	WL				
19	LC	WL	WB	BL		
Hatched	SS	WL	LC	JL	WB	BL

Values not underscored by the same line are significantly different at the level of probability indicated as follows.
Age embryo 6 days: any differences at 1% level.
Age embryo 10 days: between-two kinds at 0.5% level.
Age embryo 13 days: any differences at 1% level.
Age embryo 19 days: two differences LC-WB and LC-BL at 1% level.
Hatched: SS-LC, WL-LC, JL-BL and WB-BL at 5% level, and any others at 1% level.

significantly different from the other two with the exception of LC (Table 5). WL, however, were not different from any other kinds in 16 and 19 day embryos. At hatching the coefficients varied considerably in a wide range from 64.3 per cent for BL to 70.7 per cent for SS, which would lead to a discrepant conclusion from that of the previous researchers (4). Thus, no coefficient could be arranged on any basis as a whole, except for that a hierarchy between Leghorn and its two hybrids corresponded to the ranking by egg weight. The results confirm in general, the finding of Saeki et al. (9) in the effect of hatching egg size on the chiek weight of New Hampshires.

Simple Correlations of Egg Weight with Embryo or Chick Weight: the relationship within kinds the correlation coefficient was referred to as a product moment (Table 6). The significances were scattered in the later periods than 13 days of incubation. Nevertheless in the four kinds investigated for not less than four critical points both BL and WB indicated increasing positive correlations after 13 days of incubation while WL after 16 days of incubation, especially both WL and BL having highly positive correlations just prior to hatching time, i.e. at 19 days of incubation.

At hatching each of the four kinds indicated high positive correlation. It may suggest, however, that this statistics was different within each kind.

TABLE 6. *Correlations of Egg Weight with Embryo or Chick Weight*

Age	WL	JL	BL	SS	LC	WB
Embryo 6 days	−0.0581		0.4789		0.6469	0.5039
10	0.3066		0.5737			
13	0.5704*		0.3915		−0.8641*	0.1317
16	0.3904*		0.4570			
19	0.8531**		0.7146**		0.4942	0.4734
Hatched	0.9269**	0.8393**	0.9162**	0.6689	0.4789	0.9832**

* Significant at 5% level.
** Significant at 1% level.

Regressions of Grams of Embryo or Chick Weight on Grams of Egg Weight: in comparison of four groups investigated for more than four periods, increasing positive regressions were indicated as embryonic growth proceeded, with only two exceptions, the LC embryo at 13 days and the WB embryo at 19 days (Table 7).

Coefficients of Determination (r^2): the effect of the original egg weight on the variance of embryo weight was estimated by the coefficient of determination, as illustrated in Figure 1. This statistic was referred to by Zervas and Collins (10) and Saeki et al. (9). The coefficient indicated a variable fluctuation either between-chicken groups or between-incubation periods. The variation attributed to egg size accounted for 0.3 per cent (WL) to 41.8 per cent (LC) at 6 days of incubation

TABLE 7. *Regressions of Grams of Embryo or Chick Weight on Grams of Egg Weight.*

Age	WL	JL	BL	SS	LC	WB
Embryo 6 days	−0.0014		0.0110		0.0134	0.0053
10	0.0237		0.0419			
13	0.070**		0.066		−0.144	0.024
16	0.115*		0.378			
19	0.594**		0.559**		0.502	−0.304
Hatched	0.786**	0.516*	0.878**	0.656	0.640	0.864**

* Significant at 5% level.
** Significant at 1% level.

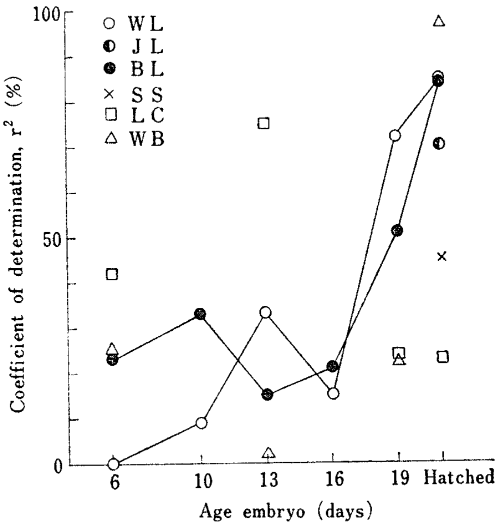

FIG. 1. The Coefficient of Determination (%) between Initial Egg Weight and Embryo or Chick Weight.

and 22.9 per cent for LC to 96.7 per cent for WB at hatching time. Thus, although widely fluctuant, the coefficient on the whole showed a positive increase with progressive incubation time.

In WL and BL investigated for all six critical points, gradual increases were observed in both groups between 6 and 10 days and rapid increases during the period from 16 days of incubation to hatching time. This statistic was found to be markedly higher, in 13 day embryo weights, 15.3 (WL) to 32.5 per cent (BL) variation, as compared with 3.00 per cent in two week embryo weight by McNary et al. (3), and only one to three per cent in 14 day embryo weight by Zervas and Collins (10). But the variation in WL embryo weight at 6 days of incubation was estimated at nearly zero while it was estimated at 0.06 per cent by the former workers at approximately corresponding periods, one week after incubation.

The variation in day-old chick attributed to egg size was 85.0 and 83.9 per cent for WL and BL respectively, which were considerably higher than 72.8 per cent for New Hampshire by Saeki et al. (9).

Various analytical procedures in statistics utilised to study the intra- and inter-breed relationship of embryonic growth to egg weight suggested, as above mentioned, that embryonic growth was more or less influenced by egg size, especially during the later periods of incubation, though the effect of egg weight on embryonic growth was somewhat obscured in this experiment since each kind consisted of the very small sample-sized materials, and since the individual embryo within each kind could be influenced by either genetically or environmentally multi-sourced factors.

Especially on unassimilated yolk weight, an appendant investigation to embryonic growth was carried out as mentioned below.

Mean Unassimilated Yolk Weight and Per Cent Unassimilated Yolk Weight of Embryo Weight: Jull and Heywang (11) in a study of the relationship of chick weight to egg weight and to the amount of yolk material present in the egg reported that the smaller the egg the smaller is the chick and the smaller is the absolute amount of 'yolk' weight in the chick at hatching time. The data in Table 8 shows unassimilated yolk weights at 16 and 19 days of incubation, and so it could reaffirm in general this statement in the original literature, again during these later incubation periods (Tables 8 and 9): 11.3 grams and 9.03 grams for WL and BL respectively, at 16 day's incubation, and from 10.03 grams for the highest ranking eggs WL to 5.19 grams for the lowest one WB at 19 days.

TABLE 8. *Mean Unassimilated Yolk Weight (Grams).*

Incubation time	WL	BL	LC	WB
16 days	11.30 ±0.27[a]	9.03 ±0.43		
19 days	10.03 ±0.31	6.15 ±0.26	5.09 ±0.41	5.19 ±0.93

a) Standard error of the mean.

TABLE 9. *Ranking and Statistical Analyses of Breeds or Crossbreds by Unassimilated Yolk Weight.*

Incubation time	Rank by unassimilated yolk weight: Large ← 1	2	3	→ Small 4
16 days	WL	BL		
19 days	WL	BL	WB	LC

Values not underscored by the same line are significantly different at the level of probability indicated as follows.

Age embryo 16 days: at 0.5% level.

Age embryo 19 days: BL-LC at 5% level, and any others at 1% level.

These earlier experimenters also found that 'yolk' weight was about 18 per cent of chick weight at hatching time. Wilson (12), in the same manner, stated that the yolk-sacs occupied approximately 15 per cent of the total mass of the chick at hatching.

On a per cent basis, [unassimilated yolk weight/(embyro- + unassimilated yolk-weight)], there was no significant difference between the two kinds at 16 day's incubation and no consistently corresponding hierarchy to different chickens in egg size at 19 days of incubation just prior to hatching (Tables 10 and 11).

TABLE 10. *Unassimilated Yolk Weight as a Percentage of Embryo Weight.*

Incubation time	WL	BL	LC	WB
16 days	40.9 ±0.7[a]	42.3 ±1.1		
19 days	25.7 ±0.7	23.2 ±1.0	20.9 ±1.5	26.4 ±4.1

a) Standard error of the mean.

TABLE 11. *Ranking and Statistical Analyses of Breeds or Crossbreds by Unassimilated Yolk Weight as a Percentage of Embryo Weight.*

Incubation time	Rank by per cent unassimilated yolk weight (Large ← → Small) 1	2	3	4
16 days	BL	WL		
19 days	WB	WL	BL	LC

Values not underscored by the same line are significantly different at the level of probability indicated as follows.
Age embryo 19 days: both WB-LC and WL-LC at 5% level.

In spite of the fact that there were, only in a few cases, significant differences at present (Table 11), the results were contrary to those of Jull and Heywang (11) who reported that although there is a significant difference in weight between the two egg size classes, the differences between the two egg size classes in respect to the mean percentage chick weight of egg weight and the mean percentage 'yolk' weight of the chick weight, respectively, are not significant. They gave that the differences in the mean percentage 'yolk' of chick weight between various pairs of hens may be due to differences in the weights of the yolks in the eggs or to different rates of assimilation of yolk material during embryonic development or to both of these factors. But this per cent measurement would give the less knowledge of yolk assimilation if one is under consideration that it is assumed, or suggested, in the general literature that some egg white passes in the yolk sac during embryogenesis.

Here again, referred to Wilson (10) that this 'yolk', together with the subcutaneous fat present in the day-old birds, brings the total percentage of available depot-food to between 20 and 23 per cent of the total dry weight of newly hatched chicks, the presently used eggs may be interested in a balance between embryo and its food supply since albumen and yolk weight, and the weight ratio of albumen to yolk varied in correlation with increased or decreased egg weight in disregard of the differences in breeds or crossbreds (Table 12).

TABLE 12. *Albumen*[a] *and Yolk Weight*[b] *and the Weight Ratio of Albumen to Yolk, Estimated on the Mean Egg Weight Basis Shown in Talbe 1. (Grams)*

	WL	JL	BL	SS	LC	WB
Albumen weight (A)	31.86	25.26	22.4	21.61	19.3	14.16
Yolk weight (B)	16.13	13.93	12.97	12.71	11.94	10.23
(A)/(B)	1.98	1.81	1.73	1.70	1.62	1.38

[a],[b] Calculated from regressions of grams of albumen or yolk weight on grams of egg weight. The regressions are presented in the previous work (5).

While it probably can be suggested that egg weight was more highly correlated with albumen than with yolk, Mizuma and Hashima (13) in a study of the influence of partial albumen removal during incubation on the embryonic development, etc., discussed that the quantitative relation between the yolk and the albumen can not be judged only from the quantity of the unabsorbed yolk measured in both groups i.e., albumen removal treatment and control, and then were led to the view that the quantity of the yolk utilised by the embryo is almost the same in the eggs whose albumen was partially removed as in the case of those in the non-treatment. Their experimental approach, i.e. albumen removal treatment during incubation, may be probably appreciated and adopted in the further study of the quantitative relation of the two major components of egg, albumen and yolk to embryonic growth or hatching as would clarify the significance of egg weight as a closed environment.

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