Change of Serum Protein of Laying Hen under Various Feeding Conditions and Egg-Laying

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Introduction

It seems probable from the observations of several workers that there exists a polymorphism in the blood serum proteins of hen. Kristjansson et al. (1963) reported that there is a possible connection between prealbumin B and early shell formation process. Lush (1963) observed fraction 8 in the serum during egg-laying, using the starch gel electrophoresis of Smithies (1955), and also fractions 12 and 13 appearing to vary inversely, that is, in the plasma of laying hens, fraction 12 is usually stronger than the other fraction. Just after hens went out of laying, fraction 13 increased to a maximum and at the same time fraction 12 decreased to a minimum. Bell and MacIndoe (1962) found no difference in the total serum protein between the laying and non-laying hens except for the pre-laying period. On the other hand, according to Rako et al. (1964) it was shown that the total serum protein level in egg-laying hens is higher than in non-laying hens, and there was a positive correlation between the serum protein level and the production of eggs. It was reported by Waldroup et al. (1965) that alpha and beta globulins and total lipoprotein in the serum are remarkably reduced by lowering the protein level of the diet. Kibe (1968) suggested that, employing liquid chromatography, fraction A is notably reduced through fasting, and fraction B showed a remarkable variance on egg-laying, and fraction F seemed to contain the characteristic protein for egg production.

This experiment was conducted to investigate the changes of the serum protein components under various feeding conditions and egg-laying by liquid chromatography and starch gel electrophoresis. In addition, egg albumen used as a sole source of protein in the semi-purified diet was also studied electrophoretically.

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Matrial and method

A 2-year-old Single Comb White Leghorn hen was used in this study. The hen was in good egg production. The compositions of semi-purified diets (10, 20% protein level and protein-free diet) are shown in Table 1. Feeds and water were provided ad libitum.

Table 1. Composition of semi-purified diets(%)

	Diet A	Diet B	Diet C
Egg albumen *	26	13	0
Dextrine	4	4	4
Corn starch	30	40.2	53.2
Fiber	5	5	5
Lime stone	5	5	5
Dicalcium phosphate	1	1	1
Mineral mix	5	5	5
Corn oil	5	5	5
Choline chloride	0.15	0.15	0.15
Glucose	18.4	21.2	21.2
Vitamin B mix	0.3	0.3	0.3
Vitamin A.D	0.15	0.15	0.15
Total	100.0	100.0	100.0
Protein level	20	10	0

* About 78% protein

The blood collection was done from the hen-fed formula ration at 1/3, 7 1/6, 16, 20 1/2, 22 3/4 and 23 3/4 hours before oviposition, and also under various feeding conditions.

The preparation of blood serum and the fractionation of serum proteins were also carried out by the method of earlier report (Kibe, 1968). Electrophoretic analysis was carried out in gels prepared with 63 g of hydrolyzed starch in 250 ml of a borate buffer. Sample slots were positioned 3 cm from the cathode end of gel. Electrophoresis was carried out for 6 hours at 10 v/cm, then the gel was stained with amido black 10 B for 10 minutes.

Results and discussion

Analysis of serum protein by liquid chromatography

Serum protein patterns progressively changing before oviposition are shown in Fig. 1. The fraction at pH ranging 4.5–4.8 that was commonly said to be

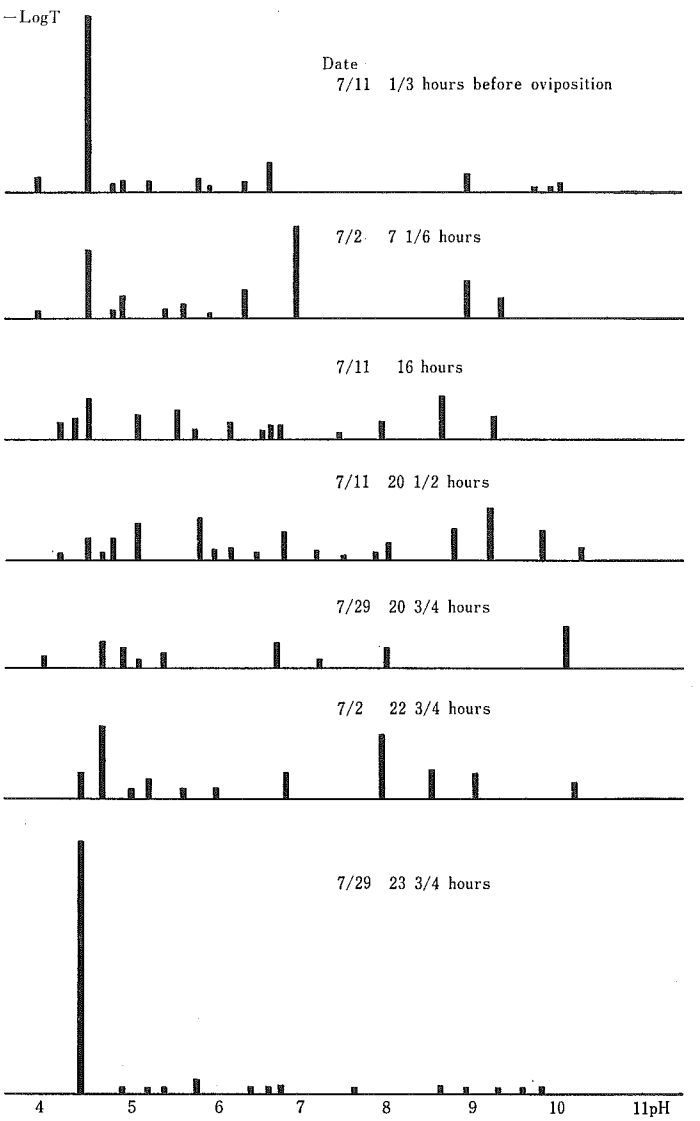

Fig. 1 Serum protein patterns of laying hen.

albumin (Akabori et al., 1960; Altman et al., 1961) remarkably increased at 1/3 hour before oviposition. This increase suggests that serum albumin may have directly influenced upon egg white secretion in the oviduct which takes place in this period as said by Warren et al. (1935). The fraction at pH 4.0 which may be glycoprotein (Akabori et al., 1960; Altman et al., 1961) appeared both in the duration of egg shell membrane formation and in the latter half of the egg white secretion. By the hypothesis of Simkiss (1961), it was suggested that the cell of the uterus secretes a substance consisting of acid mucopolysaccharide and

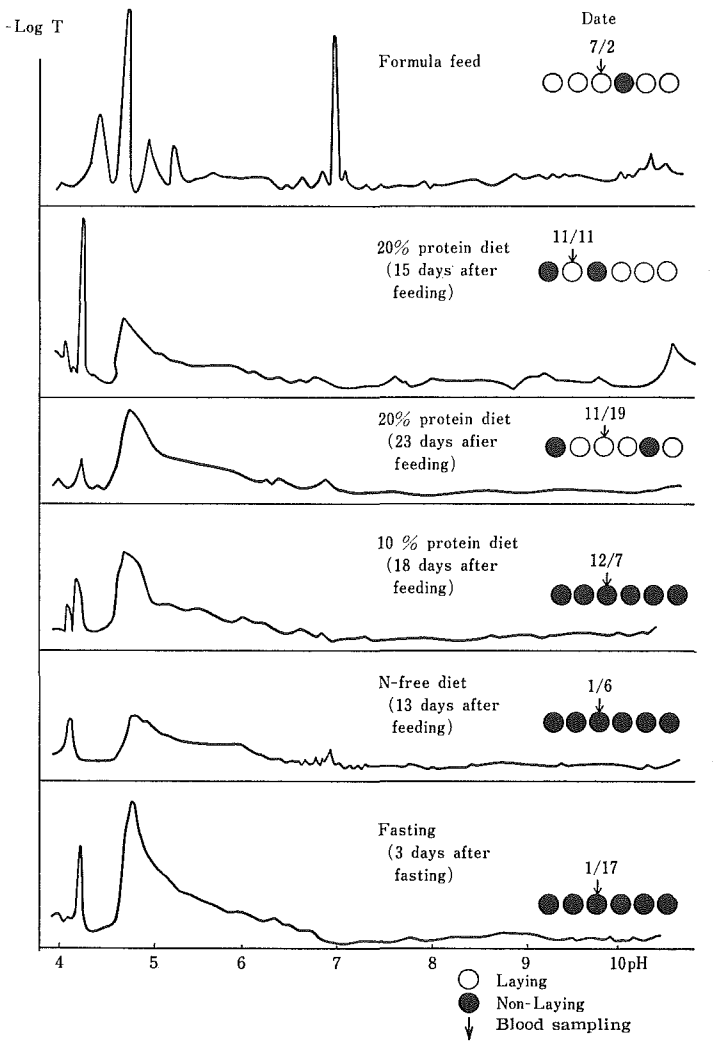

Fig. 2 Serum protein patterns of hen under various feeding conditions.

protein, and the fraction (pH 4.0) in this analysis may be related to this phenomenon. But this fraction did not appear even at 16 hours before oviposition in case of having no next ovulation.

The fraction (pH 6.3 to 7.3) which seemed to be γ-globulin (Akabori et al., 1960; Altman et al., 1961) was noted little or no change after oviposition. The fraction being in pH ranging from 8.0 to 9.4 decreased in a few hours after ovulation, if the next ovulation occurred. In the case of no ovulation it was observed that this fraction increases.

The result of the fractionation of serum proteins of hen-fed formula ration is shown in Fig. 2. Peaks shown in Fig. 2 were fractionized into 15 portions.

These figures designate that fractions 2, 3, 4, 5 and 9 appear as great peaks on the feeding of formula ration, but on the feeding of semi-purified diet (20% protein) fractions 3, 4 and 5 appear as a peak without separation and fraction 9 results in decreasing. This tendency was observed not only in the feeding of 20% protein diet but also in the feeding of 10% protein diet. This reason is not understood. On the other hand, Waldroup et al. (1965) reported that there is a tendency of dropping of α–and β–globulins in the serum of pullet fed low protein diet. Therefore, it was assumed that the protein patterns of serum were more affected by the sort of protein source than the level of protein of diet. Namely, it is considered that because the albumen (this contains about 74% of albumin) was used as a sole source of protein the effect appeared in fraction 9 which is possibly regarded as globulin fraction (Akabori et al., 1960; Altman et al., 1961). Guessing from the fact that fractions 1 and 2 are considerably affected by laying as described above, perhaps the variances of these fractions may not be much affected by the protein of diet. Fraction 2 may be possibly considered as a sort of glycoprotein from an isoelectric point (Akabori et al., 1960; Altman et al., 1961), and then, in a series of figures it is found that fraction 2 decreases in the latter half of the egg white secretion and in the shell formation process. Moreover, the appearance of fraction 1 may depend upon egg-laying, and its tendency is not obviously observed.

From the results mentioned above, it seems that the quality of the protein used as a sole source of protein (egg albumen) highly affects serum protein fractions, especially fraction 9. In this study, however, egg-laying was fairly good in the first ten days even though the 10% protein diet was provided as well as the 20% protein diet of albumen. Then, judging from serum protein patterns, the continuance in egg production seems to cease in a short time even if the dietary protein such as egg albumen is continuously fed in high level (20%). Thus, it is suggested that the serum globulin might be used as a precursor of egg yolk protein.

Electrophoretic analysis

In the course of an electrophoretic survey of the serum proteins, it was noticed that twelve fractions appeared in the serum of the feeding formula ration, and fractions 3, 4 and 5 decreased, besides fractions 6 and 8 disappeared as fed with 20% protein-containing diet. The electrograph for the protein–free diet was similar to that for the formula ration. But it was observed that the fractions below 9 in position for the protein–free diet considerably decreased in quantity in comparison with the fractions below 9 for both formula ration and 20% protein diets. The electrograph in the case of fasting resembled very much to that in the case of protein–free diet. Namely, the globulin fraction that seemed to contain

γ-globulin (Akabori et al., 1960 ; Altman et al., 1961) fairly decreased.

Observing in succession these results as a whole, it was suggested that the globulin decreased and the albumin increased under the feeding of 20% protein-containing and protein-free diets and in the state of fasting. This phenomenon corresponded to a protein source and agreed with the result afforded by liquid chromatography.

In the earlier report (Kibe, 1968), it has been described that the A/G ratio of serum protein of laying hen under fasting fairly increased. The conclusion in this experiment agreed with the above-mentioned.

As a reference, Fig. 3 shows the protein patterns of egg albumen used as a source of protein in the semi-purified diet. According to the findings of Baker et al. (1962), egg white is approximately separated into three fractions ; mucin-globulin, albumin and conalbumin. This electrograph was similarly obtained, but globulin fraction was not clearly shown in this picture. Furthermore, the investigation of inner thin, outer thin and thick albumen gave the result that lysozyme fraction (cathode end from slot, Lush, 1961) is most in the inner thin albumen

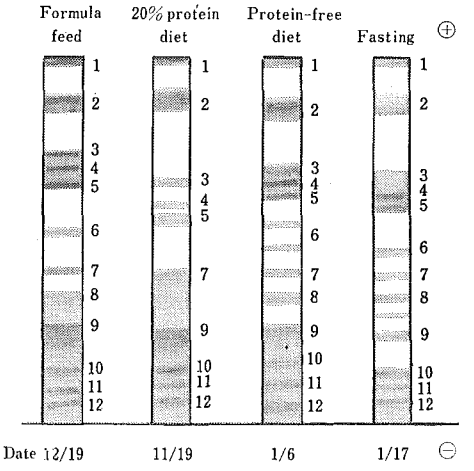

Fig. 3 Diagrams of blood sera resolved by starch gel electrophoresis.

Summary

This experiment was performed by liquid chromatography and starch gel electrophoresis in order to study alterations in the serum protein of the same hen under different feeding conditions; semi-purified diets (20% and 10% protein diet and protein free diet) and fasting.

Moreover, change of serum protein of a hen being in good egg production

at different times before oviposition was observed. Albumin in the blood serum remarkably increased just before and after oviposition. It was suggested that this may have something to concern with the formation of egg white in the oviduct. The fraction appearing at pH 4.0 may be related to egg shell membrane formation and egg white secretion. And, there was minor effect of protein source in the diet on this fraction. The twelve fractions were found in the electrograph under the feeding of formula diet. Fractions 6 and 8 disappeared as fed with 20% protein diet. The picture of electrophoresis on the feeding of protein-free diet was similar to that of formula ration. But, it was observed that the fractions below 9 in position for the protein-free diet considerably decreased in quantity in comparison with the fractions for both formula ration and 20% protein diets. Judging from the position in the picture, perhaps these fractions seemed to contain γ-globulin. These were perceptively decreased by the feeding of protein-free diet and fasting. As a whole, it was noticed that albumin and globulin fractions appear to vary with the above feeding conditions inversely. On the electrophoretical analysis of egg albumen, lysozyme fraction was found to be most in the inner thin albumen.

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飼料条件ならびに産卵後の経過時間が鶏の血清蛋白質におよぼす影響

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前報 (1968) に引きつづき，単冠白レグ産卵鶏の血清蛋白質の変動を種々の飼料条件すなわち絶食時，慣用飼料，半精製飼料（蛋白質レベル20，10および0％）給与時ならびに産卵後の経過時間によりそれぞれ調査した。なお分析方法としては液体クロマトグラフィーならびに澱粉ゲル電気泳動法を用いた。その結果を要約すれば以下のごとくである。

1. 血清アルブミンと思われるフラクションは産卵の前後においていちじるしく増加した。

2. pH4.0前後に現われる蛋白質は産卵と関係があるものと考えられ，これの蛋白質源による影響は少なかった。

3. 電気泳動法により血清蛋白質は12のフラクションに分画されたが，易動度の低いNo.9以下のフラクションは無蛋白質飼料給与時ならびに絶食時において減少する傾向がみとめられた。

4. 卵白中のリゾチーム含量は内水様卵白において最も多かった。

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