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Homeostasis as Regulated by Activated Macrophage. IX. Enhancement Effect of LPSw (a Lipopolysaccharide from Wheat Flour) on Hen Egg-Laying and Breaking Strength of Eggshell

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Oral administration of LPSw (a lipopolysaccharide from wheat flour) given at 60 μg/hen/d in drinking water, markedly enhanced eggshell strength. The monthly percentage of eggs laid with a shell strength of more than 4 kg to the total number of eggs was 32% in the group given LPSw in drinking water while it was 12% in the control group given plain water. At the same time, LPSw caused a 30% enhancement of total monthly number of eggs laid over that of control.

Keywords homeostasis; macrophage; lipopolysaccharide (LPS); wheat LPS; eggshell intensity; eggshell quality; eggshell breaking strength

Introduction

In the previous report of this series,[1] we showed that LPSw, a lipopolysaccharide from wheat flour, can activate osteoclasts and osteoblasts in chick embryonic calvaria, suggesting that it accelerates bone formation by enhancing the release and resorption of calcium. It was therefore assumed that eggshell strength would be enhanced by LPSw. This report describes this enhancement effect on hen eggshell strength.

Oral administration of LPSw increased the number of eggs with a higher density than that of control. At the same time, an unexpected enhancement was noted on the total number of eggs laid, suggesting a stimulus to ovarian hormone excretion.

Materials and Methods

Hen The breed used was DEKALB, 422 d of age at the start, with an average body weight of 1900 g. Each experimental group was composed of 6 hens, two in one open cage, and fed with standard Scott B *ad libitum*.

Supply of LPSw The LPSw used is a crude preparation, a water extract of wheat flour which was spray dried. Content of LPSw in this preparation is 0.1 mg/g as tested by Limulus reaction. The sample was dissolved in drinking water and supplied *ad libitum via* pick hole with a siphon supplier. The amount of water and of LPSw consumed was determined daily by measuring the amount of residual drinking water.

Measurement of Eggshell Breaking Strength The strength required to break an eggshell was determined using an eggshell tester made by Fujihira Ind., Tokyo. Figures are shown in kg for the intensity, indicating "bearing x kg".

Weight of Eggshell and Shell Weight per Unit (SWUSA) After shell weight was determined, SWUSA was estimated using the shell weight and a formula for oval surface area, measuring the long and short axis of each egg.

Statistical Analysis Statistical evaluation of differences between groups were made by Student's t-test or χ^2-test.

Results

Enhancement Effect of LPSw on Intensity and SWUSA of Eggshell Hens were administered LPSw dissolved in drinking water *ad libitum* and fed for 14 d. Following that period, observation was continued for 16 more days so

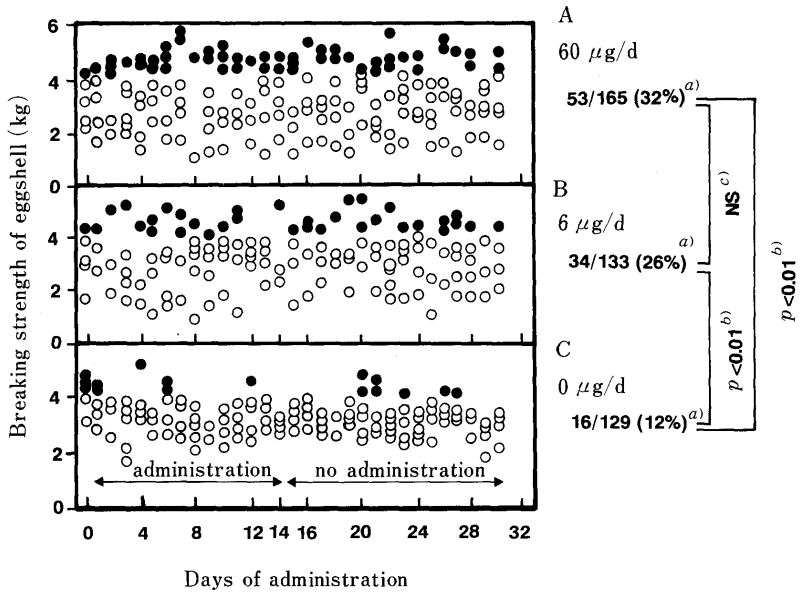

Fig. 1. Enhancement by LPSw of Eggshell Strength

Each group was consisted of 6 hens, 422 d of age, with an average body weight of 1900 g. Each circle represents the breaking strength of an eggshell. ○, egg with breaking strength of less than 4 kg; ●, egg with breaking strength of more than 4 kg. *a*) Percentage of the number of eggs with breaking strength of more than 4 kg over the total number of eggs. *b*) Statistical analysis was done by χ^2 test. *c*) NS is not significant.

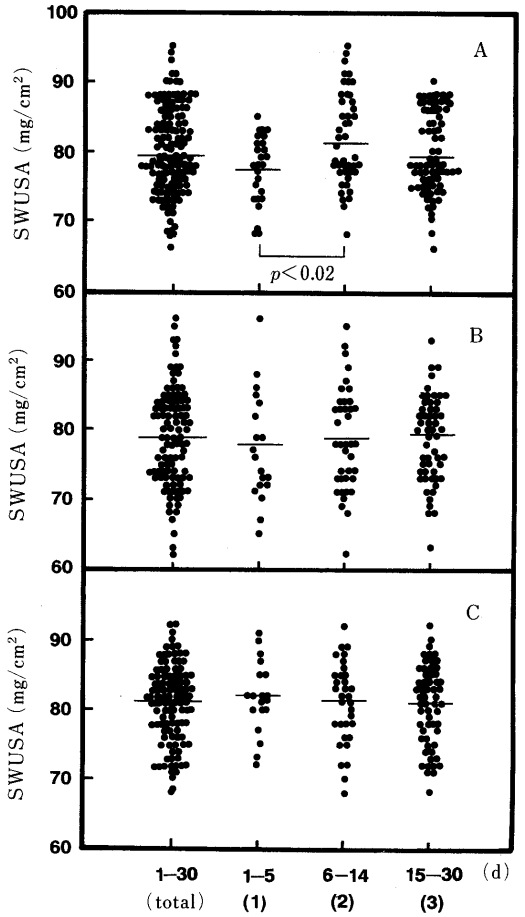

Fig. 2. Effect of LPSw on SWUSA

Based on the number of eggs in Fig. 1, SWUSA is calculated and presented by dividing the period of administration into three, (1) 1—5 d (first half), (2) 6—14 d (later half) and (3) 15—30 d (no administration). All divisions show no significant difference except for period (1) and (2) in group A ($p<0.02$). Statistical analysis was done by Student's t-test.

that the total observation period was 30 d. Breaking strength and SWUSA were followed for individual eggs. Figure 1 shows change in the strength of eggs from hens having consumed LPSw (60 μg/hen/d: group A, or 6 μg/hen/d: group B). Ten number of eggs with a breaking strength of more than 4 kg accounted for 32% of the total in group A ($p<0.01$; χ^2-test). Twenty-six percent of group B which had consumed 6 μg/hen/d showed this eggshell strength ($p<0.01$; χ^2-test). The control group, on the other hand, showed only 12%.

Figure 2 shows change of mean SWUSA values with time in each group. The 30 d test period is divided into three: (1) 1—5, (2) 6—14, and (3) 15—30 d. Period (1) represents the first half of the days treated, (2) the latter, half, and (3) no treatment for groups A or B. Group C is a control without any special treatment throughout the period. A significant increase was observed between period (1) and (2) in group A. ($p<0.02$; t-test).

Enhancing Effect of LPSw on Egg-Lay In the above experiment, we noticed that the number of eggs laid in the experimental groups were markedly enhanced. As shown in Table I the increased ratio of the total number of eggs laid in hen group A over that in the control group C was 28%. Since shell strength in this group was significantly enhanced, this means that the inner content of the egg

TABLE I. Effect of Oral Administration of LPSw on Egg-Lay of Hens

Group	A	B	C
Dose of LPSw (μg/d)	60	6	0
Number of eggs (6 hens)			
During the period of administration (14 d)	80	66	63
After cessation of administration (16 d)	85	67	66
Total	165	133	129
Increase ratio[a)]	28%	3%	0%

LPSw was administered for 14 d. Number of eggs was counted continously for 16 d after cessation of administration. Each group was composed of 6 hens.

a) Increase ratio $= \left(\frac{\text{total number of eggs in each group}}{\text{total number of eggs in group C}} - 1\right) \times 100$.

itself was also stimulated, probably due to an increase in the excretion of hormones necessary for the production as a result of LPSw uptake.

Discussion

In the previous report of this series,[1)] we reported that LPSw produced a marked stimulation of bone resorption and formation in an organ culture of chick embryonic calvaria or femur. Based on these findings, the present experiment was carried out to examine the enhancement effect of LPSw on shell intensity of hen eggs. As shown in Fig. 1, oral uptake of LPSw (60 or 6 μg/hen/d) enhanced by 32 and 26%, respectively, the number of eggs having a shell strength of more than 4 kg.

Eggs with this type of shell strength are quite valuable. Hens at 420 d of age usually lay few such eggs, as shown by the activity of the control group. Therefore, the effect of LPSw can be appreciated in two ways: one is an increase in number of shells capable of bearing 4 kg, and the other is the rejuvenation of older hens to lay such eggs.

LPSw was administered for only 14 d and yet the stimulation was maintained for 30 d. Such a prolonged effect following cessation of administration seems incredible. However, in hyperlipidemic rabbits, the suppressive effect of LPSw on serum cholesterol levels was maintained for 40 d after administration ceased.[2)] The effect of LPSw is believed to activate macrophages to regulate the homeostasis of hens constitutively, thus making such a prolonged effect possible and expectable.

SWUSA, another parameter indicative of shell strength,[3)] (Fig. 2) showed a slight increase in group A in the later period of treatment over that at the beginning. Thus, LPSw enhances eggshell weight as well as its strength. This effect on SWUSA decreased when the administration of LPSw was discontinued.

Unexpectedly, we found a larger number of eggs laid by groups A and B (Table I). As discussed earlier, the effect of LPSw may last for half a month even after its administration has been halted, as confirmed in our previous work[2)] on the suppression by LPSw of serum cholesterol levels in Watanabe heritable hyperlipidemic (WHHL) rabbit. Enhancement of egg-laying is ascribed to the greater excretion of ovarian hormones, which may, in turn, be derived from activation of macrophages by LPSw to the primed stage ready for the endogenous production of tumor necrosis factor (TNF). This also suggests the rejuvenation of older hens.

The results suggest a hopeful and productive future

in the employment of LPSw for poultry farming both quantitatively and qualitatively, not only for the number of eggs laid but also for the number with breaking strength of more than 4 kg.

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References

1) K. Kawashima, H. Endo, T. Nishizawa, H. Inagawa, T. Okutomi, A. Morikawa, G-I. Soma, and D. Mizuno, *Chem. Pharm. Bull.*, **40**, 1271 (1992).
2) T. Okutomi, T. Nishizawa, H. Inagawa, T. Takano, A. Morikawa, G-I. Soma, and D. Mizuno, *Chem. Pharm. Bull.*, **40**, 1268 (1992).
3) R. M. G. Hamilton, *Poultry Sci.*, **61**, 2022 (1982).