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Plasma Prolactin and Corticosterone Concentrations Are Changing Toward Hatch with a Different Manner Between Layer- and Broiler-Type Chickens

[1]M. Sato, [2]T. Tachibana, [3]A. Tsukada and [1]M. Furuse
[1]Laboratory of Advanced Animal and Marine Bioresources, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan
[2]Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matshyama 790-8566, Japan
[3]Department of Animal Physiology Graduate School of Bioagricultural Sciences, Nagoya University, Aichi 464-8601, Japan

Abstract: Plasma Prolactin (PRL) and Corticosterone (CORT) concentrations in broiler- and layer-type chickens were compared during embryonic development (14 and 18 days of incubation (E14 and E18)) and at hatch (P0). Plasma PRL concentrations were comparable and kept low in both types during embryonic development, but the value sharply increased at P0 with special reference to layers. Plasma CORT concentrations were similar and gradually increased toward P0 in both types and the values in layers were higher than in broilers at P0. The results obtained here suggest that plasma PRL and CORT concentrations were influenced by genetic selection just after hatching, but not during embryonic development.

Key words: Chicken, corticosterone, embryo, prolactin

INTRODUCTION

Broiler-type chickens have been intensively selected for rapid growth rate and high meat yield (Griffin and Goddard, 1994). Consequently, they grow faster than layer-type chickens which have been selected for greater egg production (Saunderson and Leslie, 1988; Mahagna and Nir, 1996; Zhao *et al*., 2004). Several factors are involved in the rapid growth of broiler chicks. For example, Hocking *et al*. (1997) reported that food intake was two-fold greater in broilers than in layer breeder males. Saito *et al*. (2005) reported that broilers were less active than layer-type chicks when exposed to an isolation-associated stress and Hocking *et al*. (1997) indicated that the proportions of time spent on preening, pecking and stereotypic pacing were greater in layers than in broiler breeders.

The rate of growth at embryonic stages also differs between broilers and layers, the growth rate of broilers being faster than that of layers (Muramatsu *et al*., 1990; Ohta *et al*., 2004; Sato *et al*., 2006a, b). However, there are few studies about the growth difference between broilers and layers during embryonic development.

The growth of muscle in various strains of chickens has been examined in terms of rates of protein synthesis, degradation and accretion using a variety of techniques (Orcutt and Young, 1982; Hentges *et al*., 1983; Maeda *et al*., 1990). All of these studies led to the conclusion that the protein deposition rate in muscles is controlled at the level of protein degradation rather than protein synthesis. Saunderson and Leslie (1988) also suggested that broiler has a faster muscle growth rate and a slower muscle degradation rate compared with layers. Corticosterone (CORT) is a major glucocorticoid in avian species. CORT has an important role in the regulation of muscle proteolysis (Ohtsuka *et al*., 1998). Marie (1981) reported that glucocorticoids appeared in the plasma on embryonic day of 12 (E12) and their concentrations rose to 10 ng mL^{-1} on 19 days of incubation (E19). In addition, Tona *et al*. (2004) indicated that plasma CORT concentrations differed in three lines of chick embryo differing growth rate from internal pipping. Therefore, the difference of plasma CORT may occur in broilers and layers if proteolysis is different in two types during embryonic development.

Ishida *et al*. (1991) suggested that mRNA encoding the Prolactin (PRL) prohormone remained low until 18 days of incubation (E18), increased on E19 and reached maximum levels on the day of hatch and changes in both pituitary and plasma concentrations of PRL closely

Corresponding Author: Mitsuhiro Furuse, Laboratory of Advanced Animal and Marine Bioresources, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

mimicking those changes in PRL mRNA levels. Intravenous injections of recombinant chicken PRL into embryo at E18 dramatically elevated plasma CORT concentrations (Kühn *et al*., 1996). Therefore, the pituitary secretory product, PRL, appeared to have positive influence on avian adrenocortical function.

In the present study, therefore, differences in plasma PRL and CORT concentrations between broilers and layers were investigated at two stages of embryonic development and on the day of hatch.

MATERIALS AND METHODS

Experimental design: Fertilized eggs of broilers and layers were purchased from, Mori hatchery, Fukuoka, Japan and Murata Hatchery, Fukuoka, Japan, respectively. All eggs were candled before incubation and only uncracked and non-broken eggs were used. All eggs were incubated at 37.6°C and RH 58-68% and turned hourly. At 14 and 18 days of incubation (E14 and E18), blood from eggs selected at random was collected into a heparinized syringe from a vein of abdominal cavity. For hatchlings, blood was collected by cardiac puncture within 2 h of hatch (P0). Blood was centrifuged at 4 °C at 9,000×g for 4 min and plasma removed and stored at -85°C until analysis.

All experimental procedures were performed according to the National Research Council, 'Guide for Care and Use of Laboratory Animals', the 'Guidance for Experiments in the Faculty of Agriculture' for the Graduate Course of Kyushu University and Law (No.105) and Notification (No.6) of the Japanese Government.

Measurement of plasma PRL concentrations: The plasma PRL concentrations were measured by radioimmunoassay at E14, E18 and P0. Briefly, chicken PRL (provided by Dr. A.F. Parlow, Pituitary Hormones and Antisera Center, National Hormone and Peptide Program, Harbor-University of California Los Angeles Medical Center, Torrance, CA) were labeled with ^{125}I by the chloramine-T method. The labeled PRL was purified by gel filtration using NAP-10 columns (Amersham Biosciences, AB, Uppsala Sweden). The plasma sample was incubated with rabbit anti-chicken PRL (source Dr. A.F. Parlow) at a final dilution of 1:400,000 and ^{125}I-labeled PRL for 48 h at 4°C. Then, goat anti-rabbitγ-globulin antiserum (Calbiochem, Merck KGaA, Darmstadt, Germany) was added and incubated for 24 h at 4°C. After a centrifugation (2600×g for 30 min) and aspiration of supernatant, the radioactivity of assay tubes was measured using a γ-counter (Aloka ARC-1000M, Aloka, Tokyo, Japan). The intra- and inter-assay variations for PRL were 8.1 and 10.1%. The number of eggs used for measurement of plasma PRL concentrations was as follows: broiler E14, 6; broiler E18, 6; broiler P0, 7; layer E14, 6; layer E18, 7; layer P0, 7.

Measurement of plasma CORT concentrations: The plasma CORT concentration at E14, E18 and P0 was measured using an enzyme immunoassay kit (Assay Designs Inc., MI, USA) at E14, E18 and P0. The number of eggs for measurement of plasma CORT concentration was as follows: Broiler E14, 6; broiler E18, 7; broiler P0, 6: Layer E14, 7; layer E18, 7; layer P0, 6. The measurement for plasma CORT was done using separate sets from plasma PRL.

Statistical analysis: Data were analyzed using a factorial two-way Analysis of Variance (ANOVA) with respect to type and developmental stage. When a significant interaction between type and stage was detected, a t-test was performed as a means separation test to compare between types within each developmental stage. Statements of significance were based on $p<0.05$. Statistical analyses were conducted using StatView (SAS, 1998). Data are expressed as means±S.E.M.

RESULTS

Comparison of plasma PRL concentrations between broilers and layers at E14, E18 and P0: Plasma PRL concentrations of broilers and layers at E14, E18 and P0 are shown in Fig. 1. Plasma PRL concentrations differed with developmental stage [$F_{(2,33)}$=48.5, $p<0.0001$]. Concentrations were low at E14 and E18 in both types, whereas they substantially increased in both broilers and layers at P0. Since a significant [$F_{(2,33)}$=3.4, $p<0.05$] interaction between stage and type was observed, a t-test was conducted to compare between types within each developmental stage. Plasma PRL concentrations tended to be higher in layers than in broilers at P0 ($p = 0.07$), but not at E14 and E18.

Comparison of plasma CORT concentrations between broilers and layers at E14, E18 and P0:Plasma CORT concentrations of broilers and layers at E14, E18 and P0 are shown in Fig. 2. The plasma concentrations of CORT gradually increased toward hatch [$F_{(2,33)}$=30.3, $p<0.0001$] in both types. There was a significant [$F_{(2,33)}$=3.6, $p<0.05$] interaction between the developmental stage and type. A t-test was conducted as a means separation test to compare between types within each developmental stage. indicated that Plasma CORT concentrations tended to be higher in layers than in broilers at P0 ($p = 0.08$), but not at E14 and E18.

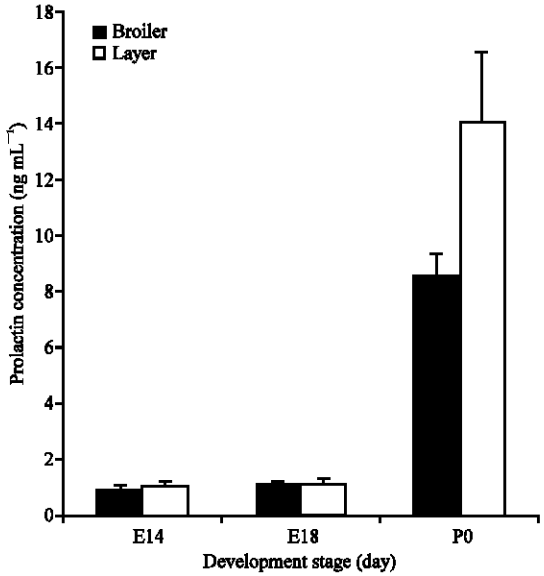

Fig. 1: The plasma prolactin concentrations of broilers and layers at E14, E18 and P0. Data are expressed as means±S.E.M

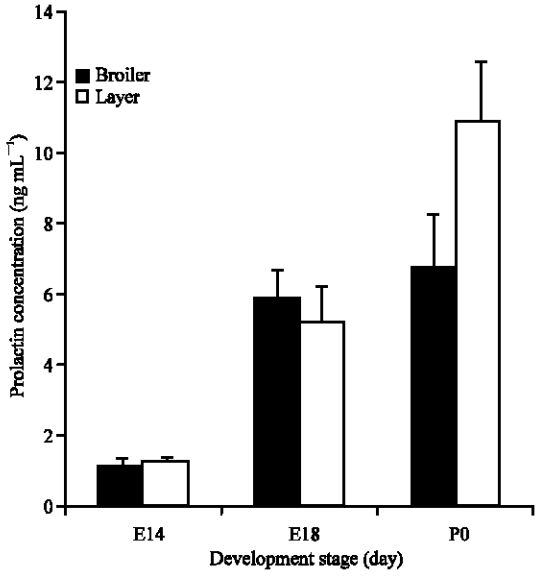

Fig. 2: The plasma corticosterone concentrations of broilers and layers at E14, E18 and P0. Data are expressed as means±S.E.M

DISCUSSION

To clarify the differences in the growth rate at embryonic stages between broilers and layers (Muramatsu *et al*., 1990; Ohta *et al*., 2004; Sato *et al*., 2006a, b), plasma PRL and CORT concentrations were compared at E14, E18 and P0 in the present study. Plasma PRL concentrations were low during embryonic development and did not differ between broilers and layers. On the other hand, plasma PRL concentrations dramatically increased at P0 and the values for layers tended to be higher than those for broilers. PRL mRNA levels were found to mimic pituitary and plasma PRL levels, being first detectable at E18 and increasing at E19 (Ishida *et al*., 1991). Therefore, plasma PRL might be remained low until E18 and increased at P0. In addition, the levels of PRL are responsive to "stress" and the plasma PRL concentrations increase after acute stress in rats (Caldeira and Franci, 2000). This result also suggests that the stress at hatching to emerge from the egg shell may be higher in layers than in broilers. This idea was confirmed by the similar to result in CORT. According to Saito *et al.* (2005) plasma CORT concentration under isolation-induced stress was significantly higher in layers than in broilers at a neonatal stage.

Yamasaki *et al.* (2003) reported that hypothalamic dopamine (PRL-release inhibiting factor) was detected in the hypothalamus during embryonic development, but the contents were not different between broilers and layers at P0. Therefore, the results obtained here were not explained by dopamine. Vasoactive Intestinal Peptide (VIP) is synthesized by the hypothalamus and released into the portal system to regulate the function of the pituitary gland (Shimatsu *et al*., 1981; Toni *et al*., 1992). Woods and Poter (1998) indicated that VIP can induce PRL secretion and lactotroph differentiation in cultured embryonic pituitary cells. These suggest that synthesis of VIP may be higher in layers than in broilers at P0. In addition, the injection of recombinant chicken PRL elevated plasma concentrations of CORT at E18 (Kühn *et al*., 1996) and Meier and Martin (1971) indicated that the daily variations in responses to CORT depend on the circadian release of endogenous pituitary RRL. In the present study, changes in plasma CORT concentrations were similar to PRL at P0. These results may suggest that PRL interacts with adrenal secretion through stimulating the hypothalamus-pituitary-adrenal axis.

CONCLUSION

In conclusion, the stress to emerge from the egg shell at hatching may be higher in layers than in broilers and layers may be models which are easily to feel stress.

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