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# Research Article
# Effect of Oestradiol-17ß on Plasma Levels of Sodium, Potassium and Chloride in the Laying Hens

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## Abstract

**Background and Objective:** Many hormones are known to take part in the control of osmoregulatory processes. One of them is oestradiol, but its role in water and electrolyte balance has not been studied much in the vertebrates. This study determined the effects of low doses of oestradiol on plasma sodium, potassium and chloride concentrations in laying hens and effects of serial blood sampling. **Materials and Methods:** Hens were given an intramuscular injection of 0.6 mg (n = 12) or 0.06 mg (n = 12) oestradiol dipropionate and the control laying hens (n = 12) were given an intramuscular injection of sterile saline. Blood samples were taken from the vena basilica immediately before the oestradiol or saline injection and then 1, 2, 3, 24 and 96 h after treatment to determine the concentration of ions. The variations of ion concentrations were examined by one-way ANOVA. **Results:** No significant differences of sodium levels were established. Concentrations of potassium decreased significantly 24 h after injection of 0.06 mg oestradiol ($p<0.04$) and saline treatment ($p<0.03$) and 96 h after injection of 0.6 mg oestradiol ($p<0.03$). Concentrations of chloride increased markedly 2 h after injection of 0.06 mg oestradiol ($p<0.002$) and saline treatment ($p<0.006$) and 24 h after injection of 0.6 mg oestradiol ($p<0.03$). A fall in the chloride levels were recorded 96 h after oestradiol ($p<0.005$) as well as saline treatment ($p<0.005$). **Conclusion:** It was concluded that, oestradiol significantly decreased the plasma potassium level 96 h after treatment ($p<0.03$) and increased the plasma chloride levels 24 h after treatment ($p<0.03$), but did not affect plasma sodium levels.

Key words: Plasma sodium level, plasma potassium level, plasma chloride level, blood sampling, oestradiol doses, laying hens

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The effect of oestradiol on electrolyte balance has not been studied much in laying hens[1]. Only the role of oestradiol in the regulation of calcium has been more extensively investigated. Oestradiol is known to cause an elevation of circulating plasma calcium levels by stimulating the hepatic synthesis of vitellogenin, a major calcium-binding protein in plasma[2]. In addition, oestradiol appears to have a direct effect on the synthesis of a uterine calcium-binding protein which may be involved in calcium transport across the uterus[3]. Oestradiol has been also considered to act on the increasing extracellular calcium utilization in the course of bone formation[4].

Several researchers have studied the effect of exogenous oestradiol on calcium plasma concentration in poultry[5-7]. An increase in circulating plasma calcium level in laying hens, 6 days after injection of 10 mg oestradiol was observed[8]. A similar elevation was found after 12 days of intramuscular injections of 2 mg oestradiol $kg^{-1}$ [9]. Total blood calcium in turkey hens increased 7 days after two injections of 75 mg oestradiol at one week interval[10]. However, high doses of oestradiol were administered in the studies[8-10]. Rezac *et al.*[11] found the significant correlations of oestradiol with cholesterol, calcium and phosphorus in laying hens. In japanese quail, the treatment of 0.2 mg oestradiol (intramuscular injection) significantly reduced the serum potassium concentration ($p<0.04$), whereas, the serum sodium concentration was slightly increased[7]. So far, little is known about the influence of small doses of estradiol on plasma electrolytes. The objective of this study was to determine the effects of oestradiol and blood sampling on plasma levels of sodium, potassium and chloride in the laying hens. The present study will advance new knowledge about the effects of oestradiol on plasma sodium, potassium and chloride levels in laying hens.

## MATERIALS AND METHODS

**Animals:** Laying hens (ISA Brown) were housed in a temperature-controlled (18°C) building in the individual laying cages (600 $cm^2$ $hen^{-1}$). The study was carried out in the battery cages for laying hens at Mendel University in Brno in July, 2015. The light regime was 15 h light and 9 h dark. Water and feed were provided *ad libitum*. Feed was a standard commercial meal (N2).

**Animal treatments:** Laying hens were given an intramuscular injection of 0.6 mg (n = 12) or 0.06 mg (n = 12) oestradiol dipropionate (Agofollin, Biotika) and control laying hens were given an intramuscular injection of sterile saline (n = 12). Treatment was conducted 3-5 h after oviposition. Blood samples were taken from the vena basilica. Blood was collected into 5 mL heparinized syringes and transferred to heparinized tubes. Samples were stored at 4°C for 1 h, then the plasma was separated by centrifugation (3000 rpm) and held at -20°C until analysis. Each animal was sampled immediately before the oestradiol or saline injection. Blood samples were also collected 1, 2, 3, 24 and 96 h after treatment to determine concentrations of ions.

**Electrolyte determination:** The sodium and potassium concentrations were determined by atomic absorption spectrophotometry (H1170, Hilger and Watt, Great Britain). The concentration of chloride was determined with an automatic analyzer (Cobas Mira S, Roche, USA)[12].

**Statistical analysis:** All results are presented as Mean±SEM. The variations of concentrations of ions were examined by one-way ANOVA and the means were compared using Student`s t-test[13]. Results were considered to be significant for $p<0.05$.

## RESULTS

**Sodium concentrations:** The patterns of sodium concentrations were similar for the oestradiol treated and control hens (Fig. 1). No significant differences in sodium levels among these groups were established.

**Potassium concentrations:** Concentrations of potassium in hens treated with 0.6 mg oestradiol did not change during the first 3 h after treatment (Fig. 2). The slight fall in potassium levels was recorded 24 h after oestradiol injection and a significant decrease was observed 96 h after treatment ($p<0.03$). Potassium concentrations in hens treated with 0.06 mg oestradiol did not change in the course of the first 3 h after treatment (Fig. 2) and then decreased significantly 24 h after injection ($p<0.04$). The lowest potassium levels were recorded 96 h after treatment ($p<0.002$). Concentrations of potassium in control hens decreased slightly within the 1st h after treatment (Fig. 2) and then increased markedly during the next hour ($p<0.002$). A slight fall in potassium levels was recorded 3 h after oestradiol injection and a significant decrease was observed 24 h after treatment ($p<0.03$). The lowest potassium concentration was established 96 h after treatment ($p<0.002$).

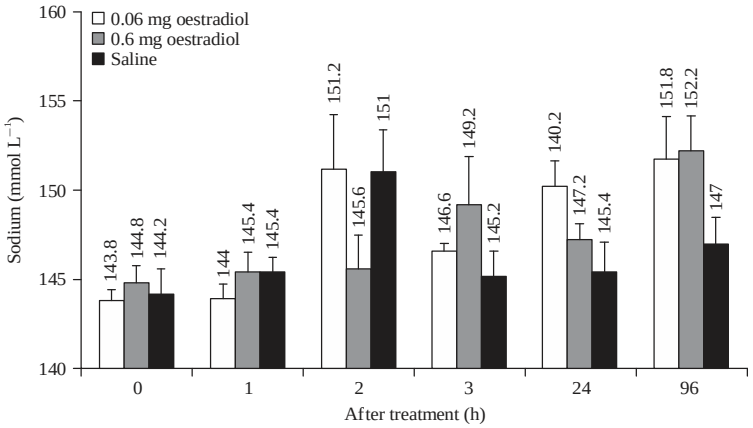


Fig. 1: Patterns of sodium concentrations (Mean±SEM) in blood plasma in oestradiol and saline-treated laying hens

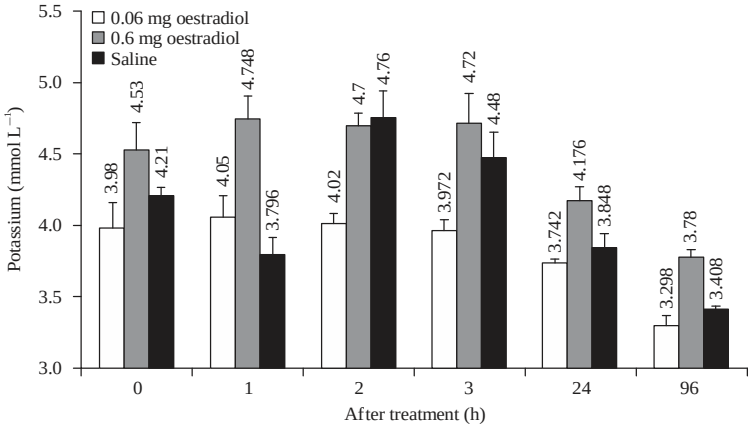


Fig. 2: Patterns of potassium concentrations (Mean±SEM) in blood plasma in oestradiol and saline-treated laying hens

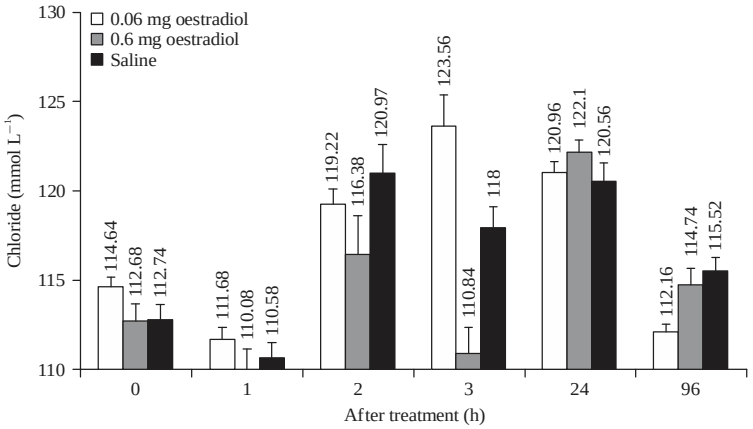


Fig. 3: Patterns of chloride concentrations (Mean±SEM) in blood plasma in oestradiol and saline-treated laying hens

**Chloride concentrations:** Concentrations of chloride in hens treated with 0.6 mg oestradiol decreased slightly 1 h after treatment (Fig. 3) and then increased to 24 h after treatment ($p<0.001$). The fall in chloride levels was recorded 96 h after oestradiol injection ($p<0.005$). Concentrations of chloride in hens treated with 0.06 mg oestradiol (Fig. 3) decreased significantly 1 h after treatment ($p<0.05$) and then increased markedly during the next hour ($p<0.002$). The

chloride concentration returned to the pre-treatment level 96 h after treatment ($p<0.001$). Concentrations of chloride in control hens decreased slightly 1 h after treatment and then increased significantly during the next hour ($p<0.006$). A fall in chloride levels was recorded 96 h after oestradiol injection ($p<0.005$).

## DISCUSSION

The effect of oestradiol on plasma levels of sodium, potassium and chloride has not been studied in laying hens. Only the role of oestradiol in the regulation of calcium has been investigated in these species. An increase in circulating plasma calcium level in laying hens 6 days after injection of 10 mg oestradiol was found[8]. A similar elevation was observed after 12 days of intramuscular injections of 2 mg oestradiol $kg^{-1}$[9].

In neither treatment did oestradiol nor blood sampling change plasma sodium levels. Similarly, blood sampling did not cause changes in plasma sodium levels in turkeys[14]. The avian hindgut and kidney are indispensable sites for electrolytes homoeostasis. The colon and coprodeum of the domestic fowl absorb sodium[15], whereas, the kidney excretes it[16]. The results suggest that blood sampling or oestradiol probably did not affect sodium homeostasis in laying hens.

In the present study, a significant effect of blood sampling on plasma levels of potassium and chloride was established. Similar results were reported for calcium[17]. The mechanism by which a rapid blood removal decreased plasma levels of potassium is not known. Potassium levels also decreased in pigeon (*Columba livia domestica*), chicken (*Gallus gallus*), peregrine falcon (*Falco peregrinus*) and turkey (*Meleagris gallopavo*) after blood sampling. These studies had a time lag that varied between 10-120 min.[12,14]. Time lag may have contributed to the significant differences in potassium levels ($p<0.05$), as potassium shifts from the plasma into the red blood cells over time. Studies with pigeon and chicken blood[12] demonstrated that a time delay between sampling and separation of cells from plasma resulted in a significant decrease in plasma potassium levels ($p<0.05$). The plasma potassium decrease with chicken blood was approximately the same when stored for 30 min as it was for pigeon blood when stored for 10 min and considerably less when compared with pigeon blood stored for 30 min.[18]. In a study with turkey blood[19], a significant decrease was noted in plasma levels of potassium, sodium and chloride ($p<0.05$). The decrease was greater as ambient temperature increased. Time from sampling to separation of cells from plasma was 2 h and lesser intervals were not studied. Regardless of the differences that may exist among avian species, the differences would be negated if centrifugation occurred immediately after blood sampling. Also, temperature of storage would not then be a factor. Plasma potassium levels are also influenced by the ambient temperature of storage and the magnitude of decrease is increased as ambient temperature increases[13].

Differences between oestradiol treated and control hens indicate that oestradiol may affect the changes of potassium levels caused by blood sampling. Next reason for this slight increase in serum sodium concentrations and the significant decrease in serum potassium concentrations ($p<0.03$) might be a result of an increase in sodium reabsorption and the potassium loss from the kidney due to the increase in serum aldosterone level. Both potassium and sodium play the important roles in the regulation of aldosterone secretion[20,21]. In order to correct pH, laying hens excrete bicarbonate through the kidneys. Bicarbonate is a negatively charged ion that needs to bind with a positively-charged ion, such as sodium or potassium, to be excreted in urine and these ions can be deficient when there is a heat stress[22]. Intestinal cells express epithelial sodium channels, which mediate sodium absorption from the intestinal lumen[23].

The effect of blood sampling on plasma chloride concentrations was possibly indirect. Plasma level of chloride was probably affected through the calcium-dependent mechanism[24-26]. It was established that calcium as the second messenger triggers and regulates intestinal chloride transport. A later significant increase of chloride level ($p<0.03$) in the group treated with a higher dose of oestradiol in comparison with the other groups suggests that oestradiol possibly is able to modulate the effect of blood sampling on plasma chloride concentrations. Oestradiol probably weakens the elevation of calcium concentrations caused by serial blood sampling.

Electrolyte balance is determined by the monovalent chemical elements (sodium, potassium and chloride), which are considered strong ions because they exert characteristic effects on the hen's acid-base balance of body fluids[27]. These ions play major roles in the synthesis of tissue proteins, maintenance of intracellular and extracellular homeostasis, maintenance of the electric potential of cell membranes, osmotic pressure and acid-base homeostasis as well as in enzyme and nerve functioning[28].

## CONCLUSION

Osmoregulatory processes play an important role in ion homeostasis, especially in the regulation of plasma sodium, potassium and chloride levels. So far, the role of oestradiol in

the control of electrolytes in the laying hens was mainly oriented on the plasma calcium level. Moreover, high doses of oestradiol were given in most studies. The results indicate that, low doses of oestradiol may decrease the plasma potassium level and increase the plasma chloride levels but not plasma sodium levels. Electrolyte balance has a crucial role in laying hen performance and it is required for acid-base balance of body fluids and litter quality. Further research will be necessary to fully understand the effects on the electrolytes in the laying hens.

## SIGNIFICANCE STATEMENT

This study discovered that oestradiol may affect plasma potassium and chloride levels in laying hens that can help to understanding the electrolyte balance. So far, only the role of oestradiol in the regulation of plasma calcium levels has been investigated in laying hens. Oestradiol is known to cause an elevation of circulating plasma calcium levels in laying hens. The present study indicates that the increased level of oestradiol can affect not only plasma calcium levels in this species but also plasma potassium and chloride levels. The electrolyte balance has a crucial role in laying hens performance and it is required for proper bone development and litter quality.

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