

From the Department of Poultry and Fur Animal Science, Agricultural University of Norway, Ås, and the Department of Physiology, Veterinary College of Norway, Oslo.

PLASMA CORTICOSTEROID LEVELS IN LAYING HENS

EFFECT OF TWO DIFFERENT BLOOD SAMPLING
TECHNIQUES AND OF ROUGH HANDLING OF THE ANIMALS

By

B. Eskeland and A. K. Blom

ESKELAND, B. and A. K. BLOM: *Plasma corticosteroid levels in laying hens. Effect of two different blood sampling techniques and of rough handling of the animals.* Acta vet. scand. 1979, 20, 270—275. — Corticosteroids were measured in blood samples collected from 10 hens in two series with a time interval of nine days. In the first series blood was collected by venipuncture (wing vein), in the second by cardiac puncture. In each series, sampling took place immediately before (control), and 5 min as well as 18 h after intentional rough handling. Only samples obtained by cardiac puncture 5 min after rough handling showed markedly elevated hormone levels.

Corticosteroids were also measured in blood collected from two other groups of hens, each including 10 birds. Samples were taken at 0 (control), 1, 5 and 40 min, one group being sampled by venipuncture, the other by cardiac puncture. None of the groups were subjected to rough handling. The hormone levels in samples obtained were significantly higher ($P < 0.005$) than the levels in the corresponding samples obtained by venipuncture.

blood sampling techniques; rough handling;
plasma corticosteroids; laying hens.

As a criterium in judging bird welfare in modern intensive poultry production, plasma corticosterone has been used and is considered to be a sensitive method (*Newcomer 1962, Nagra et al. 1963, Lei et al. 1971, Eskeland 1978*).

The importance of the technique of blood sampling is often not emphasized when plasma corticosteroid levels are measured. Cardiac puncture is widely used, because it takes short time and

gives blood in sufficient quantities. Cardiac puncture may, however, have a deleterious effect on performance, and in broilers and replacement pullets *Buckland et al.* (1974) found reduced weight gain of bled chicks compared to non-bled chicks.

The objective of this study was to compare the effect of different blood sampling techniques on plasma corticosteroid levels and to examine the effects of rough handling and of taking several blood samples.

MATERIALS AND METHODS

This study was carried out in order to compare the effect of venipuncture and cardiac puncture on plasma corticosteroid levels in one year old laying White leghorn hens. Precautions were taken not to disturb the hens before blood sampling. The sampling took place immediately after the hens were taken out of their cages. The hens used in this study were exposed to the same environment and were caged individually in cages of 40 cm × 38 cm. They were fed ad libitum of a standardized ration.

In the first part of the study 10 hens were bled by regular venipuncture in the right wing. After nine days and at the same hour of the day, the same 10 hens were bled by cardiac puncture using 5 ml heparinized syringe with a 50 mm long needle (diam. 0.8 mm).

After the control sample had been obtained either by cardiac or venipuncture, each hen was subjected to rough handling about 30 s. The birds were then put into their respective cages whereupon they were rebled after 5 min and 18 h.

In the second part of the study, series of samples were taken from two groups each of 10 hens. One group was bled by cardiac puncture, the other by venipuncture. In both groups the control sample for each hen was followed by additional sampling, 1, 5 and 40 min later, without preceding rough handling.

Corticosteroids in blood plasma were determined by competitive protein binding (*Murphy* 1967) mainly as described by *Kolanowski & Pizarro* (1969). Blood plasma from pregnant women in the last trimester was used as a source of steroid-binding protein. Free and protein-bound steroids were separated with Florisil (60–100 mesh, Sigma). All measurements were performed in duplicate and the coefficients of variation obtained were: 3.0 (mean: 3.29 ng/ml) and 3.9 (mean: 7.51 ng/ml).

RESULTS AND DISCUSSION

The results from the first part of the study are given in Table 1. The hens were bled first by venipuncture and nine days later by cardiac puncture (Table 1). Blood sampling by each of the methods took place at the same time of the day to avoid effects of possible diurnal variations. Blood sampling resulted in a mean plasma corticosteroid level of 4.5 ± 0.5 ng/ml (s.e.m.) for venipuncture and 3.9 ± 0.8 ng/ml for cardiac puncture. Out of the 10 hens bled, nine had lower plasma corticosteroid level in blood obtained by cardiac puncture than in blood taken from the vein. This may be due to the longer bleeding time necessary using venipuncture, about 3 min, versus 10 s using cardiac puncture.

Table 1. Effect of rough handling on plasma corticosteroid concentrations (ng/ml) of blood collected by venipuncture and by cardiac puncture.

Hen No.	Controls		Time after rough handling			
	(before rough handling)		5 min		18 h	
	venip.	cardiacp.	venip.	cardiacp.	venip.	cardiacp.
1	2.9	2.6	5.6	3.4	3.6	3.9
2	3.7	2.6	4.2	26.8	4.2	3.1
3	2.8	2.5	3.8	5.7	3.6	2.9
4	5.6	3.2	9.2	19.6	4.9	4.6
5	3.9	2.9	6.9	20.5	3.9	3.6
6	4.0	2.1	8.3	20.9	3.6	1.8
7	8.6	10.9	10.2	18.6	9.4	10.0
8	4.9	4.5	5.8	8.2	4.6	4.8
9	4.0	3.4	6.8	8.3	4.2	4.0
10	4.6	4.5	6.3	18.9	5.1	5.6
Mean	4.5	3.9	6.7*	15.1*	4.7	4.4
Standard error of the mean	0.5	0.8	0.6	2.5	0.5	0.7

* Significantly different from control.

Sudden release of corticosteroids caused by the handling and sampling could thus represent a strain superimposed on the environment factors to be studied. Therefore the blood samples should be collected within as short time as possible.

When frequent sampling was carried out, cardiac puncture gave a lower mean control plasma corticosteroid level (4.2 ng/

ml) than venipuncture (4.6 ng/ml) (Table 2). The difference was, however, not statistically significant ($P > 0.05$). The plasma corticosteroid level of hens bled by cardiac puncture was not significantly increased 1 min after the first blood sampling (Table 2), but was significantly increased ($P < 0.005$) 5 and 40 min after the first blood sampling.

Table 2. Plasma concentrations of corticosteroids (mean \pm s.e.m., $n = 10$) in two groups of laying hens bled by venipuncture or cardiac puncture.

	Time after first blood sampling (min)	Plasma corticosteroids (ng/ml)	
		venipuncture	cardiac puncture
First blood sampling (control)		4.6 \pm 0.3 (a) *	4.2 \pm 0.5 (e) *
2nd blood sampling	1	5.6 \pm 0.6 (b) *	4.4 \pm 0.9 (f) *
3rd blood sampling	5	7.2 \pm 0.8 (c)	17.8 \pm 3.1 (g)
4th blood sampling	40	4.9 \pm 0.9 (d) *	25.5 \pm 2.9 (h)

* No significant difference between corresponding means.
 (g-h), (c-d), $P < 0.05$.
 (a-c), $P < 0.01$.
 (b-g), (c-g), $P < 0.005$.
 (b-h), $P < 0.001$.

In peripherally bled hens, a significant increase ($P < 0.01$) in plasma corticosteroid level was observed at 5 min after the first blood sampling, but at 40 min the plasma corticosteroid level had returned almost to the initial values.

The increase in plasma corticosteroid levels from 1 to 5 to 40 min after the control sampling, using cardiac puncture, may be due to the trauma of repeated bleedings.

Rough handling of the animals followed by blood sampling 5 min later, resulted in a mean increase in plasma corticosteroids of 49 and 287 %, using venipuncture and cardiac puncture, respectively (Table 1). When no rough handling was applied, the mean percentage increases after 5 min were 56 and 324 for the two blood sampling techniques, respectively.

CONCLUSIONS

1. Cardiac puncture is a far more rapid method of blood sampling than venipuncture, the time used being 10 s and 3 min,

respectively. To minimize effects of the sampling procedure on the plasma corticosteroid level, cardiac puncture is therefore recommended when only one blood sample per animal is to be taken. On the other hand, if many samples are to be taken during a rather short period of time, venous sampling seems to be the method of choice.

2. The present data also indicate that the rise in plasma corticosteroid level brought about by the sampling procedure is not further accentuated by rough handling.

ACKNOWLEDGEMENT

The authors would like to thank Professor Dr. H. Hvidsten, the Department of Poultry and Fur Animal Science, Agricultural University of Norway, for making it possible to carry out this work.

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SAMMENDRAG

Plasma kortikosteroid nivået hos høns. Effekt av to forskjellige metoder ved blodprøvetakning og ved hardhendt behandling av dyrene.

Kortikosteroider ble målt i blodprøver fra 10 høner, tatt i to serier med ni dagers intervall. I den første serien ble blod tatt med vingevenepunktur, i den andre serien med hjertepunktur. I begge seriene

ble blodprøvene tatt like før (kontroll), 5 min og 18 timer etter hardhendt behandling. Bare blodprøven som ble tatt med hjertepunktur 5 min etter hardhendt behandling viste markert forøket hormonnivå.

Kortikosteroidnivået ble målt i blod tatt fra to andre grupper av høner, hver gruppe inneholdt 10 dyr. Prøvene ble her tatt ved tid 0 (kontroll), 1, 5 og 40 min, hvorav den ene gruppen ble tappet med venepunktur og den andre med hjertepunktur. Ingen av gruppene her ble utsatt for hardhendt behandling. Prøver tatt 5 og 40 min etter kontroll med hjertepunktur viste signifikant ($P < 0,005$) høyere hormon-nivå enn tilsvarende prøver tatt med venepunktur.

(Received November 27, 1978).

Reprints may be requested from: A. K. Blom, the Department of Physiology, Veterinary College of Norway, P. O. Box 8146 Dep., Oslo 1, Norway.