Non-invasive blood pressure measurement in the domestic chicken by the use of the Doppler principle

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Introduction

As part of a larger investigation into the effects of different anesthetic regimens in the domestic chicken, we included repeated measurements of the hemodynamic effects of the anesthetic agent over several days. The usual arterial catheter method was unsuitable for this purpose. We therefore adapted a non-invasive method using a Doppler ultrasonic flow detector, allowing repeated nontraumatic measurement of systolic blood pressure in the conscious or anesthetized bird. Systolic blood pressure was measured simultaneously in the same experiment by the Doppler principle and an invasive method in the same bird. In addition to normotensive birds, birds made hypotensive with furosemide, and birds made hypertensive with desoxycortone trimethylacetate (DOCA) were also measured.

Animals and methods

Eighteen clinically healthy female white leghorn chickens (1150-1850 g) were used. The birds were hatched on site at the animal facility from formalin disinfected embryonated eggs (The Royal Farm, Bygdøy, Oslo, Norway). They were fed a standard chicken diet and had ad-libitum access to food and water (with the exception of the DOCA group which was given 1% NaCl solution). The birds were housed singly in metal cages within the facility for the duration of the experiments. The environment within the animal room in the facility is closely controlled with respect to temperature (22C), humidity (45-55% R.H.) and light variation (12/12 light dark).

Blood pressure measurements

Invasive Systolic blood pressure was measured by an invasive procedure involving the insertion of a 0.6 mm outer diameter teflon catheter (Portex Catheters, Berck Sur Mer, France), into the brachial artery under local lidocaine anesthesia (Lidokain, xylocaine 20 mg/ml, NAF Laboratories, Oslo, Norway). The catheter was connected to a pressure transducer (HP1280C, Hewlett Packard, Waltham, Mass. USA). The signal was amplified by a carrier amplifier (HP8805B, Hewlett Packard, Waltham, Mass. USA), and recorded on a four channel thermal recorder (Hewlett Packard, Waltham, Mass. USA).

The non-invasive measurement described below was compared with invasive catherization and subsequent measurement of the systolic pressure. Blood pressure was measured simultaneously with both methods in the same bird.

Non-invasive Systolic blood pressure was measured with a non-invasive Doppler procedure. A Doppler crystal probe coated with ultrasound gel was placed over the plantar arcuate artery on the ventral side of the footweb (Fig. 1). The crystal was connected to a Doppler ultrasonic flow detector (Model 822, Parks Medical Electronics, Oregon, USA) and the detector connected to a medium gain amplifier, (HP8802, Hewlett Packard, Waltham, Mass. USA) which in turn was connected to the four channel recorder.

The bird was placed lying on its side. A commercially available inflatable polyethylene cuff, (Disposa-cuf (neonatal type), Critikon, Tampa, Fla. USA) with a width to limb circumference ratio of 0.4:1, connected to a sphygmomanometer, was used (Fig. 1). Leg feathers were not plucked. Correct placement was ascertained by locating the index line within the range demarcators inscribed on the inside of the cuff (manufacturers instruction). The pulse in the footweb was located as an au-

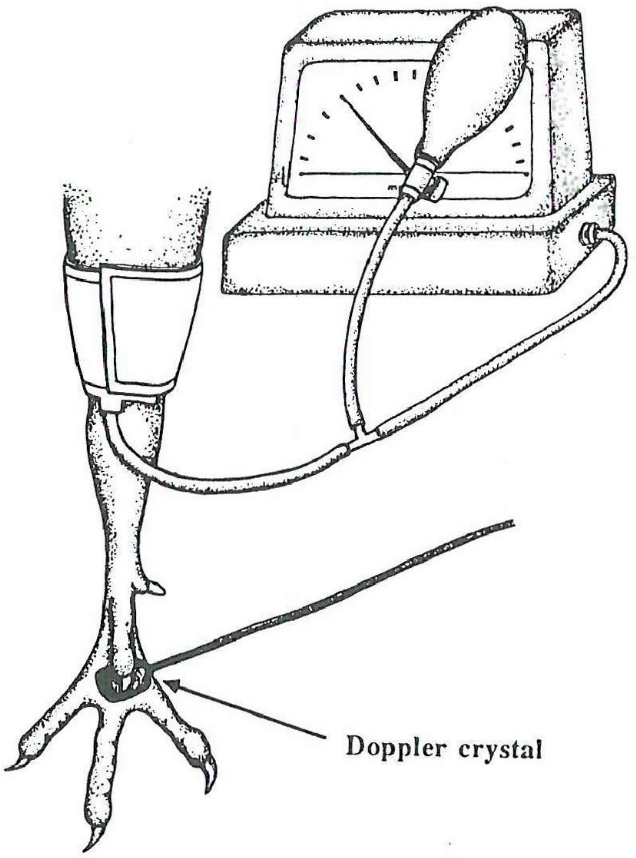

Figure 1. The leg of the chicken showing the placement of the Doppler crystal probe on the ventral side of the foot web. The arterial pulse is located using the crystal. The inflatable cuff is placed over the musculature of the distal part of the leg.

dio signal by means of the Doppler crystal probe. The cuff was inflated until the sound was extinguished. Return of the pulse sound during deflation of the cuff indicated systolic blood pressure. At least three readings were taken at

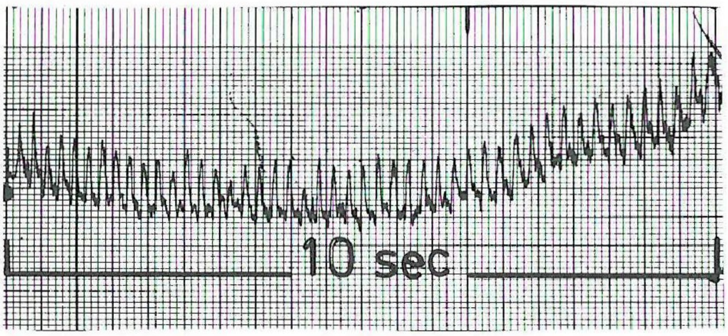

Figure 2. The thermo-trace pattern of the audio signal derived from the Doppler crystal and showing the heart rate.

each measurement. This method also enabled registration of the heart rate as an audio and written signal (Fig. 2).

Design

Hypotension

Four birds were made hypotensive by injection of furosemide (20 mg/kg) (Lasix Vet. Hoechst AG, Frankfurt, FRG) into the pectoral musculature. The birds received 3 doses at 12 hour intervals.

Normotension

Ten birds were selected at random. None had been used previously in any other procedure. Blood pressure was measured invasively and by the Doppler method as described above.

Hypertension

Four birds were made hypertensive by injection of a microcrystalline suspension of DOCA (75 mg/kg) (Percorten M. Ciba Geigy Ltd. Basle, Switzerland) into the pectoral musculature. The birds received 3 injections at 12 day intervals. They were given 1% NaCe solution as drinking water. Blood pressure was measured one week after the last injection.

Statistics

All results were analysed using a statistical analysis package (StatviewPlus™, Version 1.0, Data Metrics Inc. Philadelphia, Pa, USA). Student's *t*-test was used for paired analysis of measurements between the invasive and non-invasive groups. One way analysis of variance (ANOVA) within the groups was done according to Winer (1971). Sheffés test was used for estimation of significance at 95% confidence intervals.

Results

Measurement of cystolic blood pressure by the Doppler method was significantly (Students t-test, $P < 0.001$) lower than invasive blood pressure measurement in all three groups (25.5% in the hypo-

Table 1. Mean systolic blood pressure (mmHg± SEM) measured invasively and by the Doppler method in hypotensive (n = 4), normotensive (n = 10) and hypertensive (n = 4) chickens.

Mean systolic pressure in chickens					
	Hypotension		*Normotension*		*Hypertension*
Invasive	106 ± 4.732	N.S.	126 ± 6.272	†	186 ± 11.434
	*		*		*
Doppler	79 ± 6.250	•	110 ± 5.80	•	164 ± 7.465
	n = 4		n = 10		n = 4

* Significant (P < 0.05) difference between the invasive and Doppler measurement groups by a 2 tailed Students t-test for paired observations.

† Significant (P < 0.05) difference within the invasive measurement group by a 1 way factorial ANOVA and subsequent evaluation by Sheffés test.

• Significant (P < 0.05) difference within the Doppler measurement group by a 1 way factorial ANOVA and subsequent evaluation by Sheffés test.

N.S. represents non-significance.

tensive group, 12.7% in the normotensive group and 11.8% in the hypertensive group) (Table 1).

There was a significant (ANOVA, P < 0.001) difference in systolic blood pressure within the invasive measurement groups. There was no significant difference in systolic blood pressure measured invasively between the hypotensive and normotensive groups. The normotensive group had a significantly (Sheffé, P < 0.05) lower systolic blood pressure than the hypertensive group (Table 1).

There was a significant difference in systolic blood pressure within the Doppler measurement groups (P < 0.001, ANOVA) The hypotensive group had a significantly (P < 0.05, Sheffé) lower mean systolic blood pressure than the normotensive group. The normotensive group had a significantly (P < 0.05, Sheffé) lower systolic blood pressure than the hypertensive group (Table 1).

Figure 2 shows a typical thermo trace heart rate pattern. The heart rate in this particular case was 282 beats/min.

Discussion

Measurement of blood pressure by the Doppler principle is based on the phenomenon that ultrasonic acoustical energy is transmitted through tissues and is reflected and dispersed by acoustical interfaces in the path of the ultrasonic beam. If an acoustical interface is in motion the reflected energy received by the detector will undergo a frequency shift in direct proportion to the velocity (*Keitzer & Lichti* 1975). This is converted to an analogue signal that is amplified and expressed as a sound representing the flow of the blood and thereby the pulse of the bird.

The use of the Doppler principle to measure blood pressure and heart rate has been described in several species, including rats (*Rowberg et al.* 1969; *Bunag* 1973; *Bunag & Riley* 1974), dogs (*Harken & Smith* 1973), and man (*Hagood et al.* 1975). These authors describe a similar use of a cuff placed around an extremity and the use of audio extinction as the point of systolic blood pressure measurement. To our knowledge this method has not previously been described in birds. Adaptation of the method as described by some authors (*Rowberg et al.* 1969; *Bunag* 1973; *Bunag & Riley* 1974) required some minor modification. The form fit of the cuff is important since there has to be a constant relationship between the diameter of the leg and the width of the cuff (*Nielsen* 1982). The cuff must also conform closely to the shape of the leg.

The success of the method is dependent on a certain amount of training on the part of the operator. The quality of the audio signal is critical for a precise pressure measurement and the aural interpretation of the audio output requires some experience (*Keitzer & Lichti* 1975). The birds must be at rest and not move during reading, or this will result in disturbing noise. It was not necessary to pluck the feathers. Readings with the leg feathered were similar to those seen when the feathers were removed. Readings in the hypotensive birds was dependent on the room temperature. A higher temperature increased the peripheral circulation and made measurement easier to perform. The thermo trace pattern of the heart rate cannot be used for estimation of the cut-off point since the trace reflects a gradual reduction in the heart sound and the signal is masked by background noise before the critical cut-off point is reached.

Measurement of blood pressure by application of the Doppler principle may be assumed to be consistently lower than central invasive measurement. This difference may be ascribed to the compliance of the system consisting of the tissue of the leg and the air column in the sphygmomanometer (*Keitzer & Lichti* 1975). The application of the Doppler principle is sensitive to factors effecting local flow to the leg (ambient temperature, local vasodilation or constriction,

arteriospasm). Central invasive measurement will indicate blood pressure as a function of cardiac output at the level of the central arterial system and be less influenced by local tissue conditions.
The normotensive group had a mean blood pressure measured invasively that is within the normal range given for systolic blood pressure in chickens (*Sturkie* 1976). We obtained the same order of difference (12-13%) in blood pressure in normo- and hypertensive birds when comparing simultaneous central invasive measurement with peripheral non-invasive Doppler estimation. The approximate 13% difference we noted is of the same order as that described in rats (*Rowberg et al.* 1969; Bunag, 1973).
The larger difference between the non-invasive and invasive measurements seen in the hypotensive group can be attributed to the reduced peripheral circulation seen in this group. Furosemide is a loop diuretic. Large doses lead to hypovolemia, electrolyte changes and to elevated erythrocyte volume fraction (EVF) in chickens (*Morild et al.* 1987). This results in increased blood viscosity. Doppler measurement is sensitive to the velocity of the red cells and we assume that increased viscosity will lead to reduced flow leading to a falsely lowered central pressure estimation. Since Doppler measurement is dependent on the state of the circulation in the extremities, this will lead to a larger error if peripheral circulation is reduced in any way.
An advantage of the method is that Doppler measurement allows repeated non traumatic measurement over several days or weeks. Repeated recordings showed that any error remained constant. A disadvantage, however, is that the Doppler blood pressure measurement procedure described in this article will only give information about the systolic pressure and does not allow measurement of diastolic pressure in the conscious bird.

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The procedures described in this article have been carried out in accordance with recommendations laid down by the Norwegian State Commission for Laboratory Animals.

Summary
Non-invasive blood pressure measurement by the use of a technique using the Doppler principle allows estimation of systolic blood pressure and heart rate in the conscious bird without trauma. It permits repeated measurements over a long period with minimal stress to the bird. Blood pressure was measured in hypo-, normo-, and hypertensive birds. Measurement using the Doppler principle technique was compared with invasive catheter measurement simultaneously in the same bird.

Yhteeveto / K. Pelkonen
Doppler-periaatteeseen perustuva verenpaineen mittausmenetelmä tekee mahdolliseksi nonivasiivisesti mitata tajuisesta linnusta systolista verenpainetta ja sydämen lyöntitiheyttä vahingoittamatta lintua. Sillä voidaan pitkiä aikoja mitata toistuvasti ilman lintuun kohdistuvaa stressivaikutusta. Tutkimuksessa mitattiin verenpainetta hypo-, normo- ja hypertensiivisiltä linnuilta. Kuvattua menetelmää verrattiin invasiiviseen katetrin avulla tehtävään mittausmenetelmään tekemällä mittaukset yhtaikaisesti samasta linnusta.

Sammendrag
Non-invasiv blodtryksmåling med anvendelse af en teknik baseret på Doppler princippet gør det muligt at estimere det systoliske blodtryk og hjertefrekvensen i den vågne fugl uden traume. Teknikken tillader gentagne målinger - over et større tidsrum med minimalt stress for fugle. Blodtryk blev målt i hypo-, normo- og hypertensive fugle. Målingerne blev sammenlignet med invasive katetermålinger foretaget samtidigt i samme fugl.

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