Stunning effectiveness of broiler chickens using a two-phase stunner: pulsed direct current followed by sine wave alternating current

Effizienz der Betäubung bei Broilern bei einem Zwei-Phasen-Betäuber: Gepulster Gleichstrom gefolgt von sinussförmigem Wechselstrom

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Introduction

Stunning of chickens before slaughter is mandatory in the European Union (Council Directive 93/119). The most common stunning method in commercial chicken plants is an electrified waterbath. In the European Union high voltages are usually applied, often inducing cardiac fibrillation in the waterbath (BILGILI, 1992). Due to strong skeletal muscle contractions caused by the electrical current, this has been associated with meat quality defects such as breast muscle haemorrhaging and broken bones (GREGORY and WILKINS, 1989, 1990). In the US low voltage stunning is a common method (BILGILI, 1999). A step-up stunner of SIMMONS ENGINEERING COMPANY consists of two stunning phases: In Phase I a high frequency pulsed DC with a very low voltage of 10–15 V is applied, immediately followed by Phase II with a sine wave AC of 50 Hz. The first phase renders the birds unconscious, thus limiting muscular contractions and possible meat quality defects. In the second phase a thorough stun is induced. With very low currents used in both phases, broilers are able to recover from stunning. For welfare reasons it must be assured that the state of unconsciousness persists until death from bleeding supervenes (RAJ, 2006).

The most objective method to assess the state of (un)consciousness is EEG analysis. The occurrence of an iso-electric, flat EEG with a profound reduction to less than 10% of the pre-stun brain power has been used to indicate unconsciousness (RAJ et al., 2006). Two brain frequency bands have been considered: iso-electricity in the broader band of 2–30 Hz is indicative for overall loss of brain function, whereas the same reduction in the 13–30 Hz band has been interpreted with loss of sensibility (RAJ and O’CALLAGHAN, 2004a, b). The ‘chicken EEG clamp (CHEC)’ has been developed as a non-invasive method to EEGs of broiler chickens (COENEN et al., 2007). For quantitative comparison of brain power before and after stunning PRINZ et al. (2009) have analysed the typical base-line brain power of male and female Ross broiler chickens using the CHEC. This can now be used to calculate the relative reduction of brain power following different stunning setups. Epileptic activity with characteristic spike and wave discharges in the EEG prior to the iso-electric state has also been associated with the induction of unconsciousness in electrical waterbath stunners (SCHUTT-ABRAHAM et al., 1983).

PRINZ et al. (2010a, b) have analysed several physical parameters for the assessment of unconsciousness following high voltage waterbath stunning of broiler chickens. An increase of corneal reflexes together with the occurrence of spontaneous eye lid blinking has been identified to indicate returning consciousness (PRINZ et al., 2010a, b). Resumption of breathing will occur in birds that did not encounter cardiac arrest during stunning but is no direct indicator for consciousness and sensibility (VON WENZLAWOVICZ and VON HOLLEBEN, 2001). Severe wing flapping seems associated with convulsions rather than returning consciousness and might therefore be an indicator for meat quality problems (PRINZ et al., 2010a, b).

The aim of the present study is the assessment of the state of (un)consciousness of broiler chickens following stunning in a Simmons step-up stunner for different voltage settings in both stunning phases. Therefore the effect of the two stunning phases on the EEG and on the occurrence of physical reflexes was analysed.

Materials and methods

A total of 120 Ross 708 broiler chickens, 60 males and 60 females were raised in one flock for 7 weeks. The average weight and Standard Deviation was 2.79 ± 0.21 and 2.41 ± 0.17 for males and females respectively. For stunning a Simmons SF-7001 Pre-Stunner (SIMMONS ENGINEERING COMPANY) consisting of two stunning phases was used. In Phase I, birds receive a pulsed DC current of 550 Hz (pulse width 25%) in a shallow waterbath of about 1 cm height. The head of the chicken rested on a metal grid in the water. Water conductivity was kept constant at 40 Millisiemens/cm by the “Salt Injector Assembly”. In addition the conductivity was measured manually at the beginning and at the end of the experiment with a conductivity meter. Phase II is half as long as the first phase and consists of a metal plate immediately following the waterbath. A sine wave AC of 50 Hz is applied to the chicken’s head that rests on the metal plate.
To facilitate subsequent EEG assessment, a rotating stand fixed with a grounded metal shackle was used to immerse single broilers into the stunning cabinet and to move the broilers from Phase I to Phase II. The feet of the birds were wetted before stunning with tap water to facilitate resistance break down between the legs of the animals and the shackle. Broilers were allocated to six stunning groups, each consisting of 10 male and 10 female chickens. The first three groups received a pulsed DC (pulse width 25%) of 12 V in Phase I, with 40, 50 or 60 V sine wave AC in Phase II. Phase I was then increased to 15 V pulsed DC for the following three groups, again followed by 40, 50 or 60 V sine wave AC. Stunning time was 9.8 ± 0.9 s in Phase I and 4.9 ± 0.5 s in Phase II for all groups. A Fluke Multi-meter 189 (Fluke Corporation) was plugged into the circuit directly at the shackle to measure the rms (true root mean square) current of every broiler in Phase I. At the same position a Fluke 123 Industrial Scope Meter, 20 MHz with a Fluke 80i-110X AC/DC current probe (Fluke Corporation) measured the rms current in Phase II. All data were recorded on to a data acquisition programme Fluke View for Phase I and Fluke View SW90W for Phase II (Fluke Corporation).

Following stunning the rotating stand swung the birds towards the CHEC. Broilers were immediately fixed into the EEG device with their feet still hanging in the shackle. The EEG recording started within 10 ± 4 s post-stun and lasted for 120 s. When leaving the stunning cabinet the occurrence of tonic-clonic convulsions was assessed as described by Prinz et al. (2010a, b). Tonic-clonic convulsions were defined with a rigid backward bending of the neck and tucked wings, sometimes accompanied by small and quick muscular contractions, followed by a relaxation of the body (Prinz et al., 2010a, b). During EEG recording signs of breathing, spontaneous eye lid blinking and wing flapping were assessed and marked on observation channels on the EEG. This allows direct comparison of brain patterns with behavioural parameters. Failure of resumption of breathing was considered as a sign of cardiac failure caused by the stunning process. The occurrence of wing flapping was regarded as an indicator for severe convulsions. The corneal reflex was tested every 20 s post-stun and the results were recorded. Neck tension was also assessed, but due to the fixation of the chickens' head in the clamp, evaluation was difficult and this parameter has not been included in the further analysis. At the end of the recording period all birds were euthanised with carbon dioxide.

**Statistical Analysis**

For analysis, all EEG records were transferred to Brainvision Analyzer (Brain Products) using a Software-aid to convert Windaq-data (Dataq Instruments Inc.). The traces were filtered for the broader brain frequency band of 2–30 Hz and the smaller frequency band of 13–30 Hz and subdivided into three post-stun periods: P1 10–20 s, P2 20–30 s and P3 30–40 s. In each period, five segments of one second were marked and a Fast Fourier Transformation (FFT) calculated. From the Grand Average of the five segments, the total brain power for each of the three post-stun periods was determined. This was expressed as a percentage of the base-line brain power of an awake broiler chicken (Prinz et al., 2009). The relative brain power following the different stunning treatments was used to evaluate the level of (un)consciousness. A profound reduction of total brain power to less than 10% of the base-line EEG power was interpreted to be equivalent to an iso-electric flat EEG. A profoundly suppressed, iso-electric EEG in the 2–30 Hz band indicates failure of overall brain function, whereas the same reduction in the 13–30 Hz band was considered to be indicative for loss of sensibility. If birds showed a relative brain power of more than 10% of the base-line EEG they were considered to be inadequately stunned. In a visual assessment of the EEG recordings the occurrence of epileptiform activity was marked where the traces showed typical low frequency (2-6 Hz) spike and wave discharges (Figure 1). A characteristic chaotic EEG pattern with high amplitude and low frequency directly after stunning followed by an iso-electric EEG could be observed in many birds. This was also regarded as an indicator for a form of unconsciousness.

For statistical analysis a Nominal Logistic Regression was conducted using JMP (2007). All factor effects were calculated with the Chi-squared [$\chi^2$] Likelihood Ratio Test with Phase I Voltage [V1], Phase II Voltage [V2], sex and the interaction of V1 × V2, V1 × sex, V2 × sex and V1 × V2 × sex as fixed factors (Table 1). Body weight was included as co-variate. From the predicted values the likely percentage of birds not showing an iso-electric EEG or expressing positive behavioural reflexes was obtained and plotted.

**Results and discussion**

In both stunning phases, voltage and sex had a significant influence on the current per bird (Table 1). Male broilers obtained a significantly higher stunning current when stunned with the same voltage as compared to female broilers (Figure 2). This was not caused by deviation in live weight, as the statistical analysis was corrected for this factor. With increasing voltage the effective stunning current increased in both stunning phases. A total of 100 EEG traces were submitted to Fast Fourier Transformation (44 for males and 56 for females). 20 records could not be included due to movement artefacts and disturbances. The EEG analysis showed a significant effect of stunning voltage in Phase II (V2 AC) for the occurrence of an iso-electric EEG in the first 40 s post-stun in both brain frequency bands, the broader band of 2–30 Hz and the more limited band of 13–30 Hz (Table 1). With increasing V2 AC voltage, significantly more birds obtained an iso-electric EEG (Figure 3). For the 2–30 Hz band representing overall brain function, the interaction of voltage in Phase I (V1 DC × sex) showed a significant influence, whereas this was not significant for the 13–30 Hz band. When stunned with the same DC voltage in Phase I, significantly more male broilers showed an iso-electric EEG in the 2–30 Hz band as compared to females (Figure 3). In the three post-stun periods P1, P2 and P3, AC voltage in Phase II showed a significant effect on the occurrence of an iso-electric EEG in the 2–30 Hz band. With increasing AC voltage significantly more birds obtained a flat EEG (Figure 4). In the 13–30 Hz band this effect could only be confirmed in P1 (Table 1). In addition the interaction of V1 DC × sex showed a significant effect for the 2–30 Hz band in P1 and P3. When stunned with the same DC voltage in Phase I, in P3 significantly more male broilers obtained an iso-electric EEG compared to female birds (Figure 4).

The corneal reflex test at 20 and 40 s post-stun was significantly influenced by V1 DC and V2 AC (Table 1). Both, male and female broilers showed significantly less corneal reflexes with increasing voltage. For the later test at 40 s post-stun the interaction of V1 DC × V2 AC showed a significant effect (Table 1). The occurrence of spontaneous eye lid blinking was significantly influenced by several factors. In all three post-stun periods a significant effect of the interaction of V2 AC × sex could be detected (Table 1).
Figure 1. Examples of EEG traces of broiler chickens following stunning with the Simmons step-up stunner with three different voltage settings in Phase II sine wave AC: 40 V in the upper panel, 50 V in the middle panel and 60 V in the lower panel. The vertical bar on the left of each tracing is the Y-axis marking 80 μV, while the linear distance between two consecutive bold vertical lines along the X-axis represents 1 s.

Beispiele der EEG-Ableitung bei Brotläufern nach der Betäubung mit dem Simmons Betäuber bei drei verschiedenen Spannungen (sinusförmiger Wechselstrom) in der Phase II: obere Abbildung 40 V, mittlere Abbildung 50 V, untere Abbildung 60 V. Die vertikale Linie auf der linken Seite jedes Ableitungsdiagramms markiert die y-Achse mit 80 μV, die lineare Entfernung zwischen zwei aufeinander folgenden vertikalen Linien entlang der X-Achse entspricht 1 s.
interaction of V1 DC × sex was significant in P2 and P3 (Table 1). With the same stunning voltage male broilers showed less spontaneous eye lid blinking than females (Figure 4). Increasing V2 AC significantly suppressed the occurrence of spontaneous eyelid blinking in P2 and P3 for all animals (Figure 4). In P2 the interaction of V1 DC × sex also showed a significant effect (Table 1). Resumption of breathing was significantly influenced by sex and the interaction of V1 DC × sex proved significant (Table 1). When stunned with the same voltage in Phase I, more female broilers resumed breathing following stunning compared to males (Figure 4). The level of wing flapping was very low in all groups and no significant difference could be detected (Table 1 and Figure 5). No bird showed tonic-clonic convulsions when leaving the waterbath, all animals had a relaxed body.

A significant effect of V1 DC, sex and the interaction of V1 DC × sex could be detected for the occurrence of epileptic activity (Table 1). More female broilers expressed epileptiform activity in the EEG compared to males. Higher voltage in Phase I suppressed epileptic activity (Figure 6).

The aim of the study was the assessment of the state of (un)consciousness of broiler chickens following stunning in a Simmons step-up stunner with different voltage settings in both phases. While the voltage was kept constant within the stunning groups, female broilers obtained a significantly lower stunning current in both stunning phases indicating a higher electrical resistance (Table 1 and Figure 2). As data was corrected for live weight in the statistical analysis, this effect was not caused by the deviation in live weight. The high conductivity in the waterbath in Phase I, controlled by the Salt Injector Assembly, could not eliminate this effect. Moreover the higher resistance could also be observed in Phase II, consisting of a metal plate with direct contact to the wet chicken's head. This leads to the conclusion that the transition resistance between head and live electrode did not cause the significant difference. PRINZ et al. (2010c) observed a similar effect in a study on waterbath stunning with different electrical waveforms including sine wave AC, square wave AC and pulsed DC. They suggested the transition resistance between broiler head and waterbath as a reason for the higher resistance (RAWLES et al., 1995). Alternatively, the higher content of abdominal fat with very low conductivity in female broilers has been discussed as a reason for the higher resistance (RAWLES et al., 1995).

The analysis of stunning effectiveness was limited to the first 40 s post-stun, as the stunning process must ensure complete unconsciousness until death from bleeding supervenes. According to the instructions manual of the Simmons Pre-Stunner the sine wave AC in Phase II is responsible for a thorough stun, preventing movement of the birds during bleeding. In the present study it could be confirmed that voltage in Phase II is mainly responsible to render the birds unconscious. Increasing voltage in Phase II resulted in significantly more birds obtaining a profoundly suppressed,

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<th>Effective current Phase I</th>
<th>Voltage Phase I</th>
<th>P ≤ 0.001</th>
<th>Voltage Phase II</th>
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<th>n.s.</th>
<th>Sex</th>
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<th>V1 DC × Sex</th>
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<th>V2 AC × Sex</th>
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1 Voltage applied in Phase I: 12 V and 15 V, Voltage applied in Phase II: 40, 50 and 60 V
2 n.s.: not significant
3 Recording phases of EEG P1: 10–20 s, P2: 20–30 s, P3: 30–40 s
iso-electric EEG with less than 10% of the base-line brain power in both brain frequency bands, 2–30 Hz and 13–30 Hz (Figure 3). However, with the highest voltage setting of 60 V in the present study, more than 40% of the broilers showed inadequate stunning results in both frequency bands. This stunning setup can therefore not be recommended and further assessment of higher voltage settings is necessary to establish a minimum stunning current that ensures a stunning efficiency of 90% of the animals.

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The Simmons step-up stunner is usually used in combination with very quick and efficient bleeding. From the results of the present study it can however be observed that the level of unconsciousness is not sufficient even in the first 20 s post-stun (Figure 4). Boilers stunned with 60 V sine wave AC in Phase II show the best results with 75% of the animals obtaining isoelectricity in the EEG in P1. However, the birds show a quick recovery in the EEG In. This is in contrast to findings of Prinz et al. (2010c) on broilers stunned with 60 V sine wave AC of 50 Hz in a single stunning phase for four s. In their study more than 90% of the male birds and 89% of the female broilers maintained a profoundly suppressed, isoelectric EEG for up to 40 s post-stun. The mean rms stunning current was 72 mA and 52 mA for male and female animals respectively (Prinz et al., 2010c), thus similar to the mean rms current in the present study (Figure 4). Moreover only 20% of the male broilers in their study recovered, whereas in the present experiment 50–60% of the males resumed breathing (Figure 4). It can be assumed that this is caused by the application of the high frequent DC in Phase I, but the physiological reason remains unclear.

Positive responses to the corneal reflex test were markedly decreased when stunning voltage was increased from 12 to 15 V in Phase I (Figure 4). The occurrence of spontaneous eyelid blinking was very low in all groups with considerably less than 10% in P1 (Figure 4). Prinz et al. (2010c) found 14% of spontaneous eyelid blinking in P1 in...
well-stunned broilers after application of 60 V sine wave AC of 50 Hz for 4 s in a single phase. It can therefore be assumed that application of a high frequency pulsed DC in Phase I suppresses physical reflexes. In similar studies on single phase, high voltage stunning using either AC or DC currents, corneal reflexes and spontaneous eyelid blinking returned sooner than brain activity in recovering birds (Prinz et al., 2010a, b, c). It was therefore concluded that the occurrence of eyelid blinking indicates beginning recovery after stunning. In the present study however, the percentage of birds without iso-electric EEG is considerably higher than the number of animals expressing spontaneous eyelid blinking in all groups (Figure 4). This is obvious in the group of male broilers stunned with 15 V pulsed DC followed by 60 V AC. Although both EEG frequency bands indicate brain activity and sensibility in 45% of the animals, only 22% and 18% of the birds show a positive corneal reflex and spontaneous eyelid blinking respectively (Figure 4). It cannot be excluded that potentially conscious birds do not express physical reflexes and their assessment can therefore not be used to evaluate stunning effectiveness of the Simmons stunner. It can be assumed that the suppression of physical reflexes is influenced by the low voltage pulsed DC in Phase I. However the effect of the two stunning phases on the induction of unconsciousness and the physical appearance of the animals should be further investigated.

An epileptic EEG following waterbath stunning has been used as an indicator for unconsciousness (Schutt-Abraham et al., 1983). Raj et al. (2006) found epileptic activity in 90% of broilers stunned with a high voltage AC for 1 s. Prinz et al. (2010c) reported a lower prevalence of epileptic activity (maximum 40%) following 4 s stunning time with sine wave AC of 50 Hz. Their findings correspond
with the results of the present study, where a maximum of 30% of the birds showed epileptic activity (Figure 6) with a lower percentage in male broilers compared to females (Prinz et al., 2010c). The lower occurrence of epileptic activity of the present study compared to the findings of Raj et al. (2006) might be caused by the longer stunning time. Some epileptiform activity might have occurred already in the waterbath or during transfer of the birds to the CHEC (Prinz, 2009). Considering that the level of epileptic activity in the present study is similar to the results of single phase AC or DC stunning, it can be concluded that both stunning systems have a similar effect on the occurrence of epileptic activity. The lower level of epileptiform activity in male broilers compared to females is surprising, as one would expect a higher prevalence in males with a generally higher stunning current. Schutt-Abraham et al. (1983) described several stages of brain response following electrical stunning: a) adequately stunned: polyspike bursts (epileptiform activity) followed by a flat isoelectric line, lasting for at least 30 sec after the onset of current flow, called a complete epileptic fit. b) inadequately stunned: similar pattern as for a), but duration less than 30 sec or lack of an isoelectric line, called an incomplete epileptic fit. Based on this it might be argued that due to the lower stunning current females obtained an incomplete epileptic fit and failed to develop an isoelectric EEG pattern. Therefore the epileptiform activity was still detectable on the EEG recordings, whereas males had already passed on into isoelectricity, expressing a complete epileptic fit.

The Simmons step-up stunner aims to prevent muscle contractions, which could lead to impaired meat quality. Indeed in the present study, the bodies of the birds were relaxed with no bird showing tonic-clonic convulsions when leaving the waterbath. This is in contrast to single phase stunning with sine wave AC of 60 V for 4 s, where 75% of the birds showed tonic convulsions (Prinz et al., 2010c). Severe wing flapping has been interpreted to indicate convulsions (Prinz et al., 2010a, b). After single phase sine wave AC stunning of 60 V, 40 to 60% of the birds expressed severe wing flapping within 40 s post-stun (Prinz et al., 2010c) compared to less than 10% in the experiment presented here (Figure 5). It can be assumed that application of a low voltage pulsed DC in Phase I reduces the occurrence of convulsions. This might have a positive effect on meat quality and should be investigated in a separate study. It must however be taken into consideration that the suppression of the undesired muscular contractions occurs in response to electrical setups, which do not produce adequate unconsciousness. Further studies are required to investigate whether adequate stunning can be achieved with higher voltage settings concurrently with low levels of muscular contractions.

Conclusions

1. The voltage settings tested in the present study did not achieve unconsciousness in a sufficient number of birds. Voltages higher than 60 V AC in Phase II must therefore be applied.

2. Application of a low voltage DC in Phase I showed a suppressing effect on all physical reflexes. A number of birds with considerable brain activity in the EEG analysis did not show a corneal reflex or spontaneous eye lid blinking. Assessment of these physical reflexes can therefore be misleading for the evaluation of stunning effectiveness using the Simmons step-up stunner. Moreover the level of (un)consciousness directly following the low voltage pulsed DC in Phase I should be investigated, to ensure complete insensitivity.

3. The two-stage Simmons stunner considerably reduces convulsions and cardiac failure induced by the waterbath, which are often seen for single phase stunning using sine wave AC.

4. The low percentage of wing flapping following stunning indicates the absence of convulsions. This might have a positive effect on meat quality. It should be investigated if this positive effect can be maintained with higher voltages to ensure adequate stunning.

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Summary
Stunning efficiency of male and female broiler chickens was analysed in response to the two-phase Simmons step-up stunning. In Phase I, a pulsed DC of 550 Hz is applied in a shallow waterbath. This is immediately followed by Phase II, consisting of a metal plate with sine wave AC of 50 Hz. 120 male and female broiler chickens were randomly allocated to six stunning groups with 10 males and 10 females per group. In Phase I a voltage of 12 or 15 V was applied followed by 40, 50 or 60 V in Phase II. Stunning time was 10 and 5 s in Phase I and II respectively. The rms current per bird was recorded. To assess stunning efficiency the electroencephalogram (EEG) was recorded for 120 s post-stun. Simultaneously the occurrence of spontaneous eye lid blinking, breathing and wing flapping was assessed. The corneal reflex was tested every 20 s. The reduction of brain power was assessed in two frequency bands (2–30 Hz and 13–30 Hz) to less than 10% of the pre-stun level was analysed as indicator for adequate stunning. Female broilers showed a significantly lower rms stunning current as a result of higher electrical resistance. Phase II showed the biggest impact on stunning efficiency. Increasing voltage improved the stunning effect, but none of the analysed treatments induced unconsciousness in at least 90% of the animals. Voltage settings of more than 60 V AC in Phase II must therefore be applied. The majority of animals recovered from stunning in all groups. The occurrence of physical reflexes was suppressed in animals that were considered sensitive in the EEG analysis. Assessment of these reflexes for the evaluation of stunning efficiency can therefore not be recommended for this stunning method. No animal showed tonic-clonic convulsions following stunning and the level of severe wing flapping was very low in all groups. Meat quality advantages of this stunning method can therefore not be expected, but this must be assessed in a separate study. It must be investigated if this effect can be maintained with higher voltage settings to ensure adequate stunning efficiency.

Key words
Broiler, electroencephalogram, Simmons two-phase stunner, high frequency DC, sine wave AC

Zusammenfassung
Effizienz der Betäubung bei Broilern bei einem Zweistufen-Betäuber: Gepulster Gleichstrom gefolgt von sinusförmigem Wechselstrom


Stichworte
Broiler, Elektroenzephalogramm, Simmons Zweistufen-Betäuber, hochfrequenter Gleichstrom, sinusförmiger Wechselstrom

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FLUKE CORPORATION: Everett, WA, USA.

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Wachteln


Das Buch 'Wachteln' von Dr. Klaus Damme ist ohne Frage ein wertvolles Nachschlagewerk für Praktiker, aber auch für Hobby-Wachtelhalter. Durch die detaillierten Managementangaben bis hin zu den Tipps für die Vermarktung und die Darstellung der ökonomischen Potenziale dieser Erzeugungsrichtung hat sich dieses Buch in seiner dritten Auflage zu einem äußerst wertvollen Management-Guide entwickelt, der allen Praktikern, aber auch Hobby-Wachtelhaltern und Studierenden der Agrarwissenschaften mit Interesse an Wachteln uneingeschränkt empfohlen werden kann.