

Aanmeldingsformulier voor proeven met gewervelde dieren.

Secretariaat DEC

Aanvrager:
Afdeling:

Titel dierproef: Effects of prenatal visual and auditory stimulation on postnatal behaviour in laying hens

Aanmeldcode / Protocol: 2012071.a

Stadia van de proef:

27-08-2012	Aangemeld	[redacted]
20-09-2012	Wijzigen	Secretaris van de DEC
01-10-2012	Gekopieerd	[redacted]
03-10-2012	Gekopieerd	[redacted]

Is deze proef wetenschappelijk getoetst en goedgekeurd? Ja

Toelichting:

The research is approved and has been designed by scientists with extensive expertise in the area of egg incubation and raising chicks ([redacted]), and animal behaviour and welfare ([redacted]).

1.a. Met dit onderzoek te beantwoorden concrete vraag:

. Wetenschappelijke vraag m.b.t. van dieren

Environmental factors influence the development of chicken (*Gallus gallus domesticus*) embryo's, for example temperature and O₂ concentration (Molenaar 2010). Also, chicks are able to perceive certain stimuli already before hatching and the visual and auditory system already function in early stages of embryo development. The first differentiation of the optic vesicles starts at day E2 of incubation and chicken embryos show increased activity when exposed to light at day E3 of incubation (Rogers 1995). Furthermore, chicks can develop colour preferences after pre-hatch exposure to different colours of light (Wada et al. 1979). Thus, chicks will respond to pre-hatch light conditions.

The chick's auditory system begins to develop as soon as day E2 of incubation (Rogers 1995) and auditory sensitivity for relatively low and mid range frequency sounds (100-1500 Hz) begins at day E12 shortly after the formation of afferent synapses (Gottlieb 1968; Saunders et al. 1973; Jackson and Rubel 1978; Rubel and Fritzsch 2002; Jones et al. 2006). The exposure to sounds during incubation seems to influence the chick in later life. For example, Grier et al. (1967) found that chicks exposed to certain sounds during incubation developed a preference for those sounds compared to other sounds. Furthermore, Chaudhury et al. (2009) demonstrated that prenatal auditory stimulation causes an increase in synaptic density in the chick's central nervous system, which may lead to more efficient learning and memory.

Regarding the effects of pre-hatch light and sounds on the development of chicks, relatively little is known about the effects on behaviour in later life. Very recent Dayioglu and Ozkan (2012) showed that prenatal light exposure decrease severe and gentle feather pecking in later life in broiler chickens. The present study aims to investigate whether auditory and visual stimulation during incubation affect sociality, anxiety,

and coping strategy of young chicks, i.e. behavioural traits that are known to impact on the chick's welfare.

1.b. Het uiteindelijk doel (Maatschappelijke en wetenschappelijke relevantie):

Early life conditions, even prenatally / pre-hatch, are known to affect traits like fearfulness (or anxiety, stress susceptibility) and coping strategy, which have implications for animal production and welfare. For example, high levels of fearfulness for humans have been associated with suboptimal production in cows, pigs and chickens, and with reduced welfare. Thus, more knowledge on how early life conditions 'program' welfare determining behaviour traits could aid in optimizing keeping conditions.

Specifically, more knowledge about the influence of light and/or sounds of conspecifics during incubation on the behaviour of chickens in later life concerning sociality, anxiety, and coping style might be useful to reduce problems, such as feather pecking and stress, in the commercial laying hen industry. In current hatchery practice eggs are incubated with a lot of noise (as a result of fans and valves) and in complete darkness. With this knowledge measures can be taken to improve animal welfare by means of optimizing husbandry conditions and thus address societal concerns about egg production industry.

1.c. Lekensamenvatting:

2. Gepland vanaf: 15-10-2012 tot 31-12-2012

3. Specificatie diergroepen:

A	12	kippen	Control
B	12	kippen	Incubated with 12 h/d light exposure
C	12	kippen	Incubated with 12 h/d intermittent sounds of conspecifics
D	12	kippen	Incubated with 12h/d light plus intermittent sounds of conspecifics
E	6	kippen	target hens

4.a. Nadere aanduiding gebruikte dieren:

Commercial available Lohmann Brown laying hens will be used in this experiment.

4.b. Motivatie waarom is gekozen voor deze diersoort:

This research focuses on laying hens, for which much is known about the effects of light and sounds on their (physical) development but little about the effects on behaviour during later life.

4.c. Toelichting voor het aantal gebruikte dieren:

Literature shows that a sample size of about 12 chickens per treatment group is sufficient to obtain significant results for a range of treatment - readout parameter combinations. The number of experimental units is relatively small, but the design allows for an efficient use of degrees of freedom. For example, should interaction effects, between pre-hatch light exposure and sound exposure, prove insignificant, the number of experimental units used for estimating treatment effects increases.

We assume about half of the incubated eggs result in hens and take into account 90% fertility and 80% hatchability, but percentage of hens vary and for this reason we make calculations based on 15 (instead of 12) hens per group: $(4 \times 15) \times 2 \text{ (sex)} \times 1/0.9 \text{ (fertility)} \times 1/0.8 \text{ (hatchability)} \sim 167$ eggs. To this 6 (we take 8 for security) $\times 2 \times 1/0.9 \times 1/0.8 \sim 23$ eggs (control group without sound or light treatment) are added as to produce 6 hens ('target hens') that can function as target in sociality tests, making a total of 190 eggs.

- A. Control group: Incubated under standard conditions
- B. Light group: Incubated with 12 hours exposure to light.
- C. Sound group: Incubated with 12 hours exposure to sounds of conspecifics.
- D. Light + sound group: Incubated with 12 hours exposure to light and sounds of conspecifics.

E. Target hens,

4.d. Herkomst:

- A E. andere herkomst
- B E. andere herkomst
- C E. andere herkomst
- D E. andere herkomst
- E E. andere herkomst

Toelichting:

All eggs will be obtained from a commercial hatchery in the Netherlands.

5.a. Accommodatie:

Animals will be kept at [REDACTED]. Eggs will be incubated at the climate respiration chambers at the same accommodation.

After hatch chickens will be housed in floor pens with wood shavings (2 m²) in groups of 4 animals (one of each treatment group). A gradual change of temperature will be applied, according to the schedule advised by the breeder company. The chickens will receive a lighting schedule of light : dark hours of 16:8. Except the first 2 days post hatch, then the chickens will receive a light schedule of 23:1 (L:D), to get experienced with water and feed.

5.b. Huisvesting & Verzorging:

Animals are subject to standard care taking procedures and will be checked for health issues daily by employees of the [REDACTED] facility. Because of the young age of the hens no problems with feather pecking are expected, but protocols to deal with this are available and will be installed when necessary.

5.c. Voeding:

The animals will receive ad libitum access to feed and water. Commercial available feed for laying hens will be used.

6.a. Proefschema / proefbehandelingen:

The experiment is of a 2x2 experimental design with the incubations factors Light (no light or 12 hours of continuous light) and Sound (no sound or a 12 hour period of intermittent sound during incubation). The minimal required number of animals (hens only) per treatment is set at 12. Six additional hens ('target hens') are needed as targets in sociality tests.

Light is administered using indoor lighting bulbs, meaning an intensity in the upper range of 100 to 1000 lux (for comparison: 50,000 lux stands for average outdoor sunlight). Play back sound consist of a mixture of intra-specific sounds with an intensity in the range of 40 to 60 dB (equivalent to normal conversation at 1 m distance) and will be provided in an intermittent way (during 12 h light, 1 h on, 1 h off)

	No sounds conspecifics	Sounds conspecifics (12h)
No lighting	A	C
Lighting (12h)	B	D

A. Control group: Incubated under standard conditions: darkness, 37.8 degrees Celsius, no exposure to sounds of conspecifics or light, relative humidity = 55%, O₂ concentration = 21%, turning of the eggs will take place automatically once an hour.

B. Light group: Incubated with 12 hours exposure to light. Other circumstances are standard.

C. Sound group: Incubated with 12 hours exposure to sounds of conspecifics. Other circumstances are standard.

D. Light + sound group: Incubated with 12 hours exposure to light and sounds of conspecifics. Other circumstances are standard.

E. Target hens, treated as group A.

Hatchlings

From day E19 to E21 the hatchlings will be collected every 8 hours. They will be weighed and their appearance will be evaluated based on plumage as well as beak and leg colour. Furthermore, they will be labelled (wing tag, swift tag) per treatment group right after collection, and then they will be put in one collection box until all eggs have hatched. Except for six eggs from the control group, which will serve as testing object in the sociality tests. These chicks will be housed in a group of six immediately. The temperature of the collection box is 35 degrees Celsius. After collection chicks will get access to feed and water.

After the last chick has hatched, chicks will be randomly selected and allocated to floor pens in groups of 4.

Behaviour observations

In addition to home pen observations, a number of behaviour tests are performed. Below, maximum durations of tests are indicated, but if preliminary results show that chicks make their choices relatively rapidly, test durations will be shortened. Used tests are based on accepted (previously published) testing principles and procedures.

- Social reinstatement (measuring social motivation)

In the social reinstatement test a chick will be placed at one end of a rectangular run way (start box), while at the other end (goal box) an unfamiliar chick (one of the 'target hens') will be placed. The latency time of the first chick to reach the unfamiliar chick will be measured using a stop-watch. The duration of the test is max. 5 minutes.

- Sound preference (cross maze)

In this test a cross maze will be used to find out the chick's preference for specific sounds. A chick will be placed in one arm of the four-arm maze and given the choice between entering arms that play black either white noise (unfamiliar), maternal calls (familiar), or food calls (familiar). The duration of the test will last a maximum of 10 minutes.

- Light preference (Y-maze)

The Y-maze will be used, with on both ends of the arms a light source: on one side a light bulb of 10W and on the other side a light bulb of 1W. The latency time of making a first choice and the latency to settle will be measured, as well as the zone the chick is positioned. The duration of this test will be at most 10 minutes.

- Predator test (measurement of anxiety)

Chicks are trained (during 5 minutes) to walk to a food bowl containing meal worms. In the subsequent 'predator test' this set up is the same, but now a predator (stuffed buzzard) is placed behind the food. The latency time to reach the food is measured, and (conflict) behaviour that chicks show. Before the test the chicks are food deprived. This will be accomplished by removing the feed the morning of the test. After the test the chicks will have access to feed again. The duration of this test will be at most 5 minutes.

- Open field - exploration test (measurement of anxiety)

In the exploration test a chick will be placed in the middle of a test pen which contains patches with food particles. The latency to reach the first, second, and third food patch will be measured. Before the test the chicks will be food deprived as described with the predator test. The duration of this test will be 10 minutes.

- Cognitive bias (measurement optimism - pessimism)

In the cognitive bias test a chick will be positioned in front of a food bowl, which is filled with a food reward. After a number of successful runs to the food bowl and eating of the reward, the bowl is re-positioned and left empty. This procedure is repeated and the difference in latency to reach the food bowl when placed in these 2 different positions indicates the expectations of the chicks. Finally, the bowl is positioned in between the 2 earlier used positions. The latency to reach the bowl indicates if the chick expects a rewarding or unrewarding bowl, labelling it to be optimistic or pessimistic. Before the test the chicks will be

food deprived as described with the predator test.

- Animals are monitored for health by weighing and plumage scoring (every 2 weeks).

6.b. Mate van ongerief:

- | | |
|---|-----------------|
| A | B. Gering/Matig |
| B | B. Gering/Matig |
| C | B. Gering/Matig |
| D | B. Gering/Matig |
| E | B. Gering/Matig |

6.c. Waaruit bestaat het ongerief en hoe bent u tot uw inschatting van de mate van ongerief gekomen?

During behaviour tests, chicks are placed alone in novel test settings which initially will cause anxiety and discomfort. Being handled by humans may cause some degree of stress, but typically hens will readily habituate to this if handling is gentle and not associated with aversive experiences.

Behaviour tests are short lasting and tests that may be perceived negatively are balanced by tests that allow the chicks to explore and obtain food rewards.

Feed deprivation for a period of maximal 4 h will be considered small/moderate discomfort.

7. Welke maatregelen heeft u getroffen om het ongerief tot een minimum te beperken?

Anesthesie:

- | | |
|---|--------------------------------------|
| A | A. Niet toegepast (geen aanleiding). |
| B | A. Niet toegepast (geen aanleiding). |
| C | A. Niet toegepast (geen aanleiding). |
| D | A. Niet toegepast (geen aanleiding). |
| E | A. Niet toegepast (geen aanleiding). |

Pijnbestrijding:

- | | |
|---|--|
| A | A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat. |
| B | A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat. |
| C | A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat. |
| D | A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat. |
| E | A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat. |

To reduce the stress of handling, the chickens will be handled in a calm assured and gentle fashion. The durations of behaviour tests are short (maximum of 10 minutes) and will be shortened further where possible.

If hens suffer poor health during the study these will be taken out of the study and euthanized.

8. Toestand van dieren na einde van de proef:

- | | |
|---|---|
| A | Het dier is na de proef in leven gelaten. |
| B | Het dier is na de proef in leven gelaten. |
| C | Het dier is na de proef in leven gelaten. |
| D | Het dier is na de proef in leven gelaten. |
| E | Het dier is na de proef in leven gelaten. |

Toelichting:

Immediately after hatching male chickens not used in the experiment will be handed over to the research facilities. Furthermore, leftover female chickens will be handed over to the experimental facilities. After experimentation the used hens will also be handed over to the research facilities.

9. Welke alternatieven (vervanging, verfijning, vermindering) zijn voor de beschreven experimenten overwogen en waarom zijn deze verworpen?

Replacement:

Replacement is not possible, because this research focuses on the behaviour of laying hens. In vitro models etc. are not available for this type of experiments.

Refinement

Ill or injured animals will be removed from the test. Enrichment will be administered using wood shavings in spacious pens (4 chicks / 2 m²). The temperature will be adjusted to the needs of the chickens during development.

Reduction:

In this experiment only females are used, resulting in reduction of variation. By integrating several research questions in one experiment relatively few animals are needed. By the use of only females, variation is reduced and consequently number of used chickens could be lowered.

10. Namen van direct betrokkenen bij de dierproef (artikel 9- en 12-functionarissen):



Tabel registratiecode opties voor aanvraag 2012071.a (K14):

	1	2	3	4	5	6	7	8	9	10	11	12	13
					35	2	1	01					
A	1	51	5	12					01	1	1	2	3
B	1	51	5	12					01	1	1	2	3
C	1	51	5	12					01	1	1	2	3
D	1	51	5	12					01	1	1	2	3
E	1	51	5	6					01	1	1	2	3

Aanmeldingsformulier voor proeven met gewervelde dieren.

Secretariaat DEC

Aanvrager:
Afdeling:

Titel dierproef: Effects of prenatal visual and auditory stimulation on postnatal behaviour in laying hens

Aanmeldcode / Protocol: 2012071.b

Stadia van de proef:

03-10-2012	Aangemeld	[redacted]
04-10-2012	Wijzigen	Secretaris van de DEC
04-10-2012	Gekopieerd	[redacted]

Is deze proef wetenschappelijk getoetst en goedgekeurd? Ja

Toelichting:

The research is approved and has been designed by scientists with extensive expertise in the area of egg incubation and raising chicks ([redacted]), and animal behaviour and welfare ([redacted]). The project is approved by the professors [redacted] en [redacted].

1.a. Met dit onderzoek te beantwoorden concrete vraag:

. Wetenschappelijke vraag m.b.t. van dieren

Environmental factors influence the development of chicken (*Gallus gallus domesticus*) embryo's, for example temperature and O₂ concentration (Molenaar 2010). Also, chicks are able to perceive certain stimuli already before hatching and the visual and auditory system already function in early stages of embryo development. The first differentiation of the optic vesicles starts at day E2 of incubation and chicken embryos show increased activity when exposed to light at day E3 of incubation (Rogers 1995). Furthermore, chicks can develop colour preferences after pre-hatch exposure to different colours of light (Wada et al. 1979). Thus, chicks will respond to pre-hatch light conditions.

The chick's auditory system begins to develop as soon as day E2 of incubation (Rogers 1995) and auditory sensitivity for relatively low and mid range frequency sounds (100-1500 Hz) begins at day E12 shortly after the formation of afferent synapses (Gottlieb 1968; Saunders et al. 1973; Jackson and Rubel 1978; Rubel and Fritzsch 2002; Jones et al. 2006). The exposure to sounds during incubation seems to influence the chick in later life. For example, Grier et al. (1967) found that chicks exposed to certain sounds during incubation developed a preference for those sounds compared to other sounds. Furthermore, Chaudhury et al. (2009) demonstrated that prenatal auditory stimulation causes an increase in synaptic density in the chick's central nervous system, which may lead to more efficient learning and memory.

Regarding the effects of pre-hatch light and sounds on the development of chicks, relatively little is known about the effects on behaviour in later life. Very recent Dayiolglu and Ozkan (2012) showed that prenatal light exposure decrease severe and gentle feather pecking in later life in broiler chickens. The present study aims to investigate whether auditory and visual stimulation during incubation affect sociality, anxiety,

and coping strategy of young chicks. The study aims to detect possible effects on behaviour, focussing on those that relate to welfare and share a regulation by the serotonin system. Tryptamine-signalling, like that of serotonin and melatonin, is under the influence of light-exposure, making the selected behaviour likely candidates to respond to experimental treatments.

1.b. Het uiteindelijke doel (Maatschappelijke en wetenschappelijke relevantie):

Early life conditions, even prenatally / pre-hatch, are known to affect traits like fearfulness (or anxiety, stress susceptibility) and coping strategy, which have implications for animal production and welfare. For example, high levels of fearfulness for humans have been associated with suboptimal production in cows, pigs and chickens, and with reduced welfare. Thus, more knowledge on how early life conditions 'program' welfare determining behaviour traits could aid in optimizing keeping conditions.

Specifically, more knowledge about the influence of light and/or sounds of conspecifics during incubation on the behaviour of chickens in later life concerning sociality, anxiety, and coping style might be useful to reduce problems, such as feather pecking and stress, in the commercial laying hen industry. In current hatchery practice eggs are incubated with a lot of noise (as a result of fans and valves) and in complete darkness. With this knowledge measures can be taken to improve animal welfare by means of optimizing husbandry conditions and thus address societal concerns about egg production industry.

1.c. Leksamenvatting:

2. Gepland vanaf: 15-10-2012 tot 31-12-2012

3. Specificatie diergroepen:

A	12	kippen	Control
B	12	kippen	Incubated with 12 h/d light exposure
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D	12	kippen	Incubated with 12h/d light plus intermittent sounds of conspecifics
E	6	kippen	target hens

4.a. Nadere aanduiding gebruikte dieren:

Commercial available Lohmann Brown laying hens will be used in this experiment.

4.b. Motivatie waarom is gekozen voor deze diersoort:

This research focuses on laying hens, for which much is known about the effects of light and sounds on their (physical) development but little about the effects on behaviour during later life.

4.c. Toelichting voor het aantal gebruikte dieren:

A range of behaviour parameters is measured and Principal Component Analyses are used to integrate raw score for multiple behaviours into traits that are represented by component scores. The latter are normally distributed and expressed with a mean of 0 and a standard deviation of 1, with the majority of scores typically ranging between -5 and +5. A minimal detectable contrasts of 1 (10% on the scale of measurement) would imply 20 units per treatment, i.e. when accepting a type I error (alpha) of 0.05 (2-sided) and type II error of 0.1 (power=0.9) and performing a one-way ANOVA (one factor with 2 levels). For 10 units this per treatment this would result in a minimal detectable contrasts of 1.5 or 15%. For this study, the range of 10-15% difference is considered biologically relevant and should be revealed as a significant difference, setting the range of acceptable number of animals per treatment on 10 to 20. The study design is multifactorial and multiple measures per chickens allow the use of mixed model, and this means that statistical procedures are expected to have more power than the described one-way ANOVA. For this reason the actual number of animals per treatment (combination) is selected to be near the lower threshold, namely 12. Thus, the number of experimental units allows for an efficient use of degrees of freedom. For example, should interaction effects, between pre-hatch light exposure and sound exposure,

proof insignificant, the number of experimental units used for estimating treatment effects increases. Also, literature shows that a sample size of about 12 chickens per treatment group is sufficient to obtain significant results for a range of treatment - readout parameter combinations.

We assume about half of the incubated eggs result in hens and take into account 90% fertility and 80% hatchability, but percentage of hens vary and for this reason we make calculations based on 15 (instead of 12) hens per group: $(4 \times 15) \times 2$ (sex) $\times 1/0.9$ (fertility) $\times 1/0.8$ (hatchability) ~ 167 eggs. To this 6 (we take 8 for security) $\times 2 \times 1/0.9 \times 1/0.8 \sim 23$ eggs (control group without sound or light treatment) are added as to produce 6 hens ('target hens') that can function as target in sociality tests, making a total of 190 eggs.

- A. Control group: Incubated under standard conditions
- B. Light group: Incubated with 12 hours exposure to light.
- C. Sound group: Incubated with 12 hours exposure to sounds of conspecifics.
- D. Light + sound group: Incubated with 12 hours exposure to light and sounds of conspecifics.
- E. Target hens,

4.d. Herkomst:

- A E. andere herkomst
- B E. andere herkomst
- C E. andere herkomst
- D E. andere herkomst
- E E. andere herkomst

Toelichting:

All eggs will be obtained from a commercial hatchery in the Netherlands.

5.a. Accommodatie:

Animals will be kept at [REDACTED]. Eggs will be incubated at the climate respiration chambers at the same accommodation.

After hatch chickens will be housed in floor pens with wood shavings (2 m²) in groups of 4 animals (one of each treatment group). A gradual change of temperature will be applied, according to the schedule advised by the breeder company. The chickens will receive a lighting schedule of light : dark hours of 16:8. Except the first 2 days post hatch, then the chickens will receive a light schedule of 23:1 (L:D), to get experienced with water and feed.

5.b. Huisvesting & Verzorging:

Animals are subject to standard care taking procedures and will be checked for health issues daily by employees of the [REDACTED] facility. Because of the young age of the hens no problems with feather pecking are expected, but protocols to deal with this are available and will be installed when necessary.

5.c. Voeding:

The animals will receive ad libitum access to feed and water. Commercial available feed for laying hens will be used.

6.a. Proefschema / proefbehandelingen:

The experiment is of a 2x2 experimental design with the incubations factors Light (no light or 12 hours of continuous light) and Sound (no sound or a 12 hour period of intermittent sound during incubation). The minimal required number of animals (hens only) per treatment is set at 12. Six additional hens ('target hens') are needed as targets in sociality tests.

Light is administered using indoor lighting bulbs, meaning an intensity in the upper range of 100 to 1000 lux (for comparison: 50,000 lux stands for average outdoor sunlight). The light exposure during incubation is administered by a conventional white bulb as used in an earlier study (Protocol:2009077.b) and following the earlier work Rogers and Workman (Appl. Anim. Behav Sci. 1989), who used 40W bulbs producing 500-600 lux. This should provide sufficient contrast with the control treatment, which involves

eggs being incubated in darkness.

Play back sound consist of a mixture of intra-specific sounds with an intensity in the range of 40 to 60 dB (equivalent to normal conversation at 1 m distance) and will be provided in an intermittent way (during 12 h light, 1 h on, 1 h off)

	No sounds conspecifics	Sounds conspecifics (12h)
No lighting	A	C
Lighting (12h)	B	D

A. Control group: Incubated under standard conditions: darkness, 37.8 degrees Celsius, no exposure to sounds of conspecifics or light, relative humidity = 55%, O₂ concentration = 21%, turning of the eggs will take place automatically once an hour.

B. Light group: Incubated with 12 hours exposure to light. Other circumstances are standard.

C. Sound group: Incubated with 12 hours exposure to sounds of conspecifics. Other circumstances are standard.

D. Light + sound group: Incubated with 12 hours exposure to light and sounds of conspecifics. Other circumstances are standard.

E. Target hens, treated as group A.

Hatchlings

From day E19 to E21 the hatchlings will be collected every 8 hours. They will be weighed and their appearance will be evaluated based on plumage as well as beak and leg colour. Furthermore, they will be labelled (wing tag, swift tag) per treatment group right after collection, and then they will be put in one collection box until all eggs have hatched. Except for six eggs from the control group, which will serve as testing object in the sociality tests. These chicks will be housed in a group of six immediately. The temperature of the collection box is 35 degrees Celsius. After collection chicks will get access to feed and water.

After the last chick has hatched, chicks will be randomly selected and allocated to floor pens in groups of 4.

Behaviour observations

In addition to home pen observations, a number of behaviour tests are performed. Below, maximum durations of tests are indicated, but if preliminary results show that chicks make their choices relatively rapidly, test durations will be shortened. Used tests are based on accepted (previously published) testing principles and procedures.

- Social reinstatement (measuring social motivation)

In the social reinstatement test a chick will be placed at one end of a rectangular run way (start box), while at the other end (goal box) an unfamiliar chick (one of the 'target hens') will be placed. The latency time of the first chick to reach the unfamiliar chick will be measured using a stop-watch. The duration of the test is max. 5 minutes.

- Sound preference (cross maze)

In this test a cross maze will be used to find out the chick's preference for specific sounds. A chick will be placed in one arm of the four-arm maze and given the choice between entering arms that play black either white noise (unfamiliar), maternal calls (familiar), or food calls (familiar). The duration of the test will last a maximum of 10 minutes.

- Light preference (Y-maze)

The Y-maze will be used, with on both ends of the arms a light source: on one side a light bulb of 10W and on the other side a light bulb of 1W. The latency time of making a first choice and the latency to settle will be measured, as well as the zone the chick is positioned. The duration of this test will be at most 10 minutes.

- Predator test (measurement of anxiety)

Chicks are trained (during 5 minutes) to walk to a food bowl containing meal worms. In the subsequent 'predator test' this set up is the same, but now a predator (stuffed buzzard) is placed behind the food. The latency time to reach the food is measured, and (conflict) behaviour that chicks show. Before the test the chicks are food deprived. This will be accomplished by removing the feed the morning of the test. After the test the chicks will have access to feed again. The duration of this test will be at most 5 minutes.

- Open field - exploration test (measurement of anxiety)

In the exploration test a chick will be placed in the middle of a test pen which contains patches with food particles. The latency to reach the first, second, and third food patch will be measured. Before the test the chicks will be food deprived as described with the predator test. The duration of this test will be 10 minutes.

- Cognitive bias (measurement optimism - pessimism)

In the cognitive bias test a chick will be positioned in front of a food bowl, which is filled with a food reward. After a number of successful runs to the food bowl and eating of the reward, the bowl is re-positioned and left empty. This procedure is repeated and the difference in latency to reach the food bowl when placed in these 2 different positions indicates the expectations of the chicks. Finally, the bowl is positioned in between the 2 earlier used positions. The latency to reach the bowl indicates if the chick expects a rewarding or unrewarding bowl, labelling it to be optimistic or pessimistic. Before the test the chicks will be food deprived as described with the predator test.

- Animals are monitored for health by weighing and plumage scoring (every 2 weeks).

Hens from a same pen may influence each other and for this reason the groups are restricted in size and replicated. Possible, 'pen-effects' are accounted for in the statistical procedures (including pen in the random component of the model).

Time Schedule

Experimental (age) week2: Sound preference (cross maze)
week3 Light preference (Y-maze)
week4 Open field - exploration test (measurement of anxiety)
week5 Predator test (measurement of anxiety)
week6 Social reinstatement (measuring social motivation)
week7 Cognitive bias (measurement optimism - pessimism)

6.b. Mate van ongerief:

A	B. Gering/Matig
B	B. Gering/Matig
C	B. Gering/Matig
D	B. Gering/Matig
E	B. Gering/Matig

6.c. Waaruit bestaat het ongerief en hoe bent u tot uw inschatting van de mate van ongerief gekomen?

During behaviour tests, chicks are placed alone in novel test settings which initially will cause anxiety and discomfort. Being handled by humans may cause some degree of stress, but typically hens will readily habituate to this if handling is gentle and not associated with aversive experiences.

Behaviour tests are short lasting and tests that may be perceived negatively are balanced by tests that allow the chicks to explore and obtain food rewards.

Feed deprivation for a period of maximal 4 h, which occurs 3 times, is considered to cause small/moderate discomfort.

7. Welke maatregelen heeft u getroffen om het ongerief tot een minimum te beperken?

Anesthesie:

- A A. Niet toegepast (geen aanleiding).
- B A. Niet toegepast (geen aanleiding).
- C A. Niet toegepast (geen aanleiding).
- D A. Niet toegepast (geen aanleiding).
- E A. Niet toegepast (geen aanleiding).

Pijnbestrijding:

- A A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- B A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- C A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- D A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- E A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.

To reduce the stress of handling, the chickens will be handled in a calm assured and gentle fashion. The durations of behaviour tests are short (maximum of 10 minutes) and will be shortened further where possible.

If hens suffer poor health during the study these will be taken out of the study and euthanized. Poor health implies, for example, a feather score of 3 or 4 (on a scale of 0 -4 , Bright A, TA Jones and MS Dawkins 2006 Animal Welfare 15:113-118:), or a score of 2 for longer than 2 weeks. Several days of significant poor body condition and general weakness (behavioural apathy) is also a reason for removing hens from the study. In this, the care takers have the lead in daily checking the hens' health and indicating when hens are to be euthanized.

8. Toestand van dieren na einde van de proef:

- A Het dier is na de proef in leven gelaten.
- B Het dier is na de proef in leven gelaten.
- C Het dier is na de proef in leven gelaten.
- D Het dier is na de proef in leven gelaten.
- E Het dier is na de proef in leven gelaten.

Toelichting:

Immediately after hatching male chickens not used in the experiment will be handed over to the research facilities. Furthermore, leftover female chickens will be handed over to the experimental facilities. After experimentation the used hens will also be handed over to the research facilities. The latter implies that hens may be maintained at [REDACTED] for production purposes or passed on to private people to live on as a companion animal, but if such is not feasible the animals are euthanized.

9. Welke alternatieven (vervanging, verfijning, vermindering) zijn voor de beschreven experimenten overwogen en waarom zijn deze verworpen?

Replacement:

Replacement is not possible, because this research focuses on the behaviour of laying hens. In vitro models etc. are not available for this type of experiments.

Refinement

Ill or injured animals will be removed from the test. Enrichment will be administered using wood shavings in spacious pens (4 chicks / 2 m²). The temperature will be adjusted to the needs of the chickens during development.

Reduction:

By integrating several research questions in one experiment relatively few animals are needed.

10. Namen van direct betrokkenen bij de dierproef (artikel 9- en 12-functionarissen):

[REDACTED]



Tabel registratiecode opties voor aanvraag 2012071.b (K14):

	1	2	3	4	5	6	7	8	9	10	11	12	13
					35	2	1	01					
A	1	51	5	12					01	1	1	2	3
B	1	51	5	12					01	1	1	2	3
C	1	51	5	12					01	1	1	2	3
D	1	51	5	12					01	1	1	2	3
E	1	51	5	6					01	1	1	2	3

Aanmeldingsformulier voor proeven met gewervelde dieren.

Secretariaat DEC

Aanvrager:
Afdeling:

Titel dierproef: Effects of prenatal visual and auditory stimulation on postnatal behaviour in laying hens

Aanmeldcode / Protocol: 2012071.c

Stadia van de proef:

04-10-2012	Aangemeld		
04-10-2012	Positief advies na behandeling DEC		Secretaris van de DEC
22-11-2012	Proef gestart		
16-01-2013	Opmerkingen		
14-02-2013	Welzijnsevaluatie aangemaakt		
14-02-2013	Welzijnsevaluatie aangemeld		
14-02-2013	Welzijnsevaluatie goedgekeurd		

Is deze proef wetenschappelijk getoetst en goedgekeurd? Ja

Toelichting:

The research is approved and has been designed by scientists with extensive expertise in the area of egg incubation and raising chicks (), and animal behaviour and welfare (). The project is approved by the professors en

1.a. Met dit onderzoek te beantwoorden concrete vraag:

. Wetenschappelijke vraag m.b.t. van dieren

Environmental factors influence the development of chicken (*Gallus gallus domesticus*) embryo's, for example temperature and O₂ concentration (Molenaar 2010). Also, chicks are able to perceive certain stimuli already before hatching and the visual and auditory system already function in early stages of embryo development. The first differentiation of the optic vesicles starts at day E2 of incubation and chicken embryos show increased activity when exposed to light at day E3 of incubation (Rogers 1995). Furthermore, chicks can develop colour preferences after pre-hatch exposure to different colours of light (Wada et al. 1979). Thus, chicks will respond to pre-hatch light conditions.

The chick's auditory system begins to develop as soon as day E2 of incubation (Rogers 1995) and auditory sensitivity for relatively low and mid range frequency sounds (100-1500 Hz) begins at day E12 shortly after the formation of afferent synapses (Gottlieb 1968; Saunders et al. 1973; Jackson and Rubel 1978; Rubel and Fritsch 2002; Jones et al. 2006). The exposure to sounds during incubation seems to influence the chick in later life. For example, Grier et al. (1967) found that chicks exposed to certain sounds during incubation developed a preference for those sounds compared to other sounds. Furthermore, Chaudhury et al. (2009) demonstrated that prenatal auditory stimulation causes an increase in synaptic density in the chick's central nervous system, which may lead to more efficient learning and memory.

Regarding the effects of pre-hatch light and sounds on the development of chicks, relatively little is known about the effects on behaviour in later life. Very recent Dayiolglu and Ozkan (2012) showed that prenatal light exposure decrease severe and gentle feather pecking in later life in broiler chickens. The present study aims to investigate whether auditory and visual stimulation during incubation affect sociality, anxiety, and coping strategy of young chicks. The study aims to detect possible effects on behaviour, focussing on those that relate to welfare and share a regulation by the serotonin system. Tryptamine-signalling, like that of serotonin and melatonin, is under the influence of light-exposure, making the selected behaviour likely candidates to respond to experimental treatments.

1.b. Het uiteindelijke doel (Maatschappelijke en wetenschappelijke relevantie):

Early life conditions, even prenatally / pre-hatch, are known to affect traits like fearfulness (or anxiety, stress susceptibility) and coping strategy, which have implications for animal production and welfare. For example, high levels of fearfulness for humans have been associated with suboptimal production in cows, pigs and chickens, and with reduced welfare. Thus, more knowledge on how early life conditions 'program' welfare determining behaviour traits could aid in optimizing keeping conditions.

Specifically, more knowledge about the influence of light and/or sounds of conspecifics during incubation on the behaviour of chickens in later life concerning sociality, anxiety, and coping style might be useful to reduce problems, such as feather pecking and stress, in the commercial laying hen industry. In current hatchery practice eggs are incubated with a lot of noise (as a result of fans and valves) and in complete darkness. With this knowledge measures can be taken to improve animal welfare by means of optimizing husbandry conditions and thus address societal concerns about egg production industry.

1.c. Leksamenvatting:

2. Gepland vanaf: 15-10-2012 tot 31-12-2012

3. Specificatie diergroepen:

A	12	kippen	Control
B	12	kippen	Incubated with 12 h/d light exposure
C	12	kippen	Incubated with 12 h/d intermittent sounds of conspecifics
D	12	kippen	Incubated with 12h/d light plus intermittent sounds of conspecifics
E	6	kippen	target hens

4.a. Nadere aanduiding gebruikte dieren:

Commercial available Lohmann Brown laying hens will be used in this experiment.

4.b. Motivatie waarom is gekozen voor deze diersoort:

This research focuses on laying hens, for which much is known about the effects of light and sounds on their (physical) development but little about the effects on behaviour during later life.

4.c. Toelichting voor het aantal gebruikte dieren:

A range of behaviour parameters is measured and Principal Component Analyses are used to integrate raw score for multiple behaviours into traits that are represented by component scores. The latter are normally distributed and expressed with a mean of 0 and a standard deviation of 1, with the majority of scores typically ranging between -5 and +5. A minimal detectable contrasts of 1 (10% on the scale of measurement) would imply 20 units per treatment, i.e. when accepting a type I error (alpha) of 0.05 (2-sided) and type II error of 0.1 (power=0.9) and performing a one-way ANOVA (one factor with 2 levels). For 10 units this per treatment this would result in a minimal detectable contrasts of 1.5 or 15%. For this study, the range of 10-15% difference is considered biologically relevant and should be revealed as a significant difference, setting the range of acceptable number of animals per treatment on 10 to 20. The study design is multifactorial and multiple measures per chickens allow the use of mixed model, and this

means that statistical procedures are expected to have more power than the described one-way ANOVA. For this reason the actual number of animals per treatment (combination) is selected to be near the lower threshold, namely 12. Thus, the number of experimental units allows for an efficient use of degrees of freedom. For example, should interaction effects, between pre-hatch light exposure and sound exposure, prove insignificant, the number of experimental units used for estimating treatment effects increases. Also, literature shows that a sample size of about 12 chickens per treatment group is sufficient to obtain significant results for a range of treatment - readout parameter combinations.

We assume about half of the incubated eggs result in hens and take into account 90% fertility and 80% hatchability, but percentage of hens vary and for this reason we make calculations based on 15 (instead of 12) hens per group: $(4 \times 15) \times 2$ (sex) $\times 1/0.9$ (fertility) $\times 1/0.8$ (hatchability) ~ 167 eggs. To this 6 (we take 8 for security) $\times 2 \times 1/0.9 \times 1/0.8 \sim 23$ eggs (control group without sound or light treatment) are added as to produce 6 hens ('target hens') that can function as target in sociality tests, making a total of 190 eggs.

- A. Control group: Incubated under standard conditions
- B. Light group: Incubated with 12 hours exposure to light.
- C. Sound group: Incubated with 12 hours exposure to sounds of conspecifics.
- D. Light + sound group: Incubated with 12 hours exposure to light and sounds of conspecifics.
- E. Target hens,

4.d. Herkomst:

- A E. andere herkomst
- B E. andere herkomst
- C E. andere herkomst
- D E. andere herkomst
- E E. andere herkomst

Toelichting:

All eggs will be obtained from a commercial hatchery in the Netherlands.

5.a. Accommodatie:

Animals will be kept at [REDACTED]. Eggs will be incubated at the climate respiration chambers at the same accommodation.

After hatch chickens will be housed in floor pens with wood shavings (2 m²) in groups of 4 animals (one of each treatment group). A gradual change of temperature will be applied, according to the schedule advised by the breeder company. The chickens will receive a lighting schedule of light : dark hours of 16:8. Except the first 2 days post hatch, then the chickens will receive a light schedule of 23:1 (L:D), to get experienced with water and feed.

5.b. Huisvesting & Verzorging:

Animals are subject to standard care taking procedures and will be checked for health issues daily by employees of the [REDACTED] facility. Because of the young age of the hens no problems with feather pecking are expected, but protocols to deal with this are available and will be installed when necessary.

5.c. Voeding:

The animals will receive ad libitum access to feed and water. Commercial available feed for laying hens will be used.

6.a. Proefschema / proefbehandelingen:

The experiment is of a 2x2 experimental design with the incubations factors Light (no light or 12 hours of continuous light) and Sound (no sound or a 12 hour period of intermittent sound during incubation). The minimal required number of animals (hens only) per treatment is set at 12. Six additional hens ('target hens') are needed as targets in sociality tests.

Light is administered using indoor lighting bulbs, meaning an intensity in the upper range of 100 to 1000

lux (for comparison: 50,000 lux stands for average outdoor sunlight). The light exposure during incubation is administered by a conventional white bulb as used in an earlier study (Protocol:2009077.b) and following the earlier work Rogers and Workman (Appl. Anim. Behav Sci. 1989), who used 40W bulbs producing 500-600 lux. This should provide sufficient contrast with the control treatment, which involves eggs being incubated in darkness.

Playback sound consist of a mixture of intra-specific sounds with an intensity in the range of 40 to 60 dB (equivalent to normal conversation at 1 m distance) and will be provided in an intermittent way (during 12 h light, 1 h on, 1 h off)

	No sounds conspecifics	Sounds conspecifics (12h)
No lighting	A	C
Lighting (12h)	B	D

A. Control group: Incubated under standard conditions: darkness, 37.8 degrees Celsius, no exposure to sounds of conspecifics or light, relative humidity = 55%, O₂ concentration = 21%, turning of the eggs will take place automatically once an hour.

B. Light group: Incubated with 12 hours exposure to light. Other circumstances are standard.

C. Sound group: Incubated with 12 hours exposure to sounds of conspecifics. Other circumstances are standard.

D. Light + sound group: Incubated with 12 hours exposure to light and sounds of conspecifics. Other circumstances are standard.

E. Target hens, treated as group A.

Hatchlings

From day E19 to E21 the hatchlings will be collected every 8 hours. They will be weighed and their appearance will be evaluated based on plumage as well as beak and leg colour. Furthermore, they will be labelled (wing tag, swift tag) per treatment group right after collection, and then they will be put in one collection box until all eggs have hatched. Except for six eggs from the control group, which will serve as testing object in the sociality tests. These chicks will be housed in a group of six immediately. The temperature of the collection box is 35 degrees Celsius. After collection chicks will get access to feed and water.

After the last chick has hatched, chicks will be randomly selected and allocated to floor pens in groups of 4.

Behaviour observations

In addition to home pen observations, a number of behaviour tests are performed. Below, maximum durations of tests are indicated, but if preliminary results show that chicks make their choices relatively rapidly, test durations will be shortened. Used tests are based on accepted (previously published) testing principles and procedures.

- Social reinstatement (measuring social motivation)

In the social reinstatement test a chick will be placed at one end of a rectangular run way (start box), while at the other end (goal box) an unfamiliar chick (one of the 'target hens') will be placed. The latency time of the first chick to reach the unfamiliar chick will be measured using a stop-watch. The duration of the test is max. 5 minutes.

- Sound preference (cross maze)

In this test a cross maze will be used to find out the chick's preference for specific sounds. A chick will be placed in one arm of the four-arm maze and given the choice between entering arms that playback either white (broad-band) noise (unfamiliar), maternal calls (familiar), or food calls (familiar). The duration of the test will last a maximum of 10 minutes.

- Light preference (Y-maze)

The Y-maze will be used, with on both ends of the arms a light source: on one side a light bulb of 10W and on the other side a light bulb of 1W. The latency time of making a first choice and the latency to settle

will be measured, as well as the zone the chick is positioned. The duration of this test will be at most 10 minutes.

- Predator test (measurement of anxiety)

Chicks are trained (during 5 minutes) to walk to a food bowl containing meal worms. In the subsequent 'predator test' this set up is the same, but now a predator (stuffed buzzard) is placed behind the food. The latency time to reach the food is measured, and (conflict) behaviour that chicks show. Before the test the chicks are food deprived. This will be accomplished by removing the feed the morning of the test. After the test the chicks will have access to feed again. The duration of this test will be at most 5 minutes.

- Open field - exploration test (measurement of anxiety)

In the exploration test a chick will be placed in the middle of a test pen which contains patches with food particles. The latency to reach the first, second, and third food patch will be measured. Before the test the chicks will be food deprived as described with the predator test. The duration of this test will be 10 minutes.

- Cognitive bias (measurement optimism - pessimism)

In the cognitive bias test a chick will be positioned in front of a food bowl, which is filled with a food reward. After a number of successful runs to the food bowl and eating of the reward, the bowl is re-positioned and left empty. This procedure is repeated and the difference in latency to reach the food bowl when placed in these 2 different positions indicates the expectations of the chicks. Finally, the bowl is positioned in between the 2 earlier used positions. The latency to reach the bowl indicates if the chick expects a rewarding or unrewarding bowl, labelling it to be optimistic or pessimistic. Before the test the chicks will be food deprived as described with the predator test.

- Animals are monitored for health by weighing and plumage scoring (every 2 weeks).

Hens from a same pen may influence each other and for this reason the groups are restricted in size and replicated. Possible, 'pen-effects' are accounted for in the statistical procedures (including pen in the random component of the model).

Time Schedule

Experimental (age) week2: Sound preference (cross maze)
week3 Light preference (Y-maze)
week4 Open field - exploration test (measurement of anxiety)
week5 Predator test (measurement of anxiety)
week6 Social reinstatement (measuring social motivation)
week7 Cognitive bias (measurement optimism - pessimism)

6.b. Mate van ongerief:

A	B. Gering/Matig
B	B. Gering/Matig
C	B. Gering/Matig
D	B. Gering/Matig
E	B. Gering/Matig

6.c. Waaruit bestaat het ongerief en hoe bent u tot uw inschatting van de mate van ongerief gekomen?

During behaviour tests, chicks are placed alone in novel test settings which initially will cause anxiety and discomfort. Being handled by humans may cause some degree of stress, but typically hens will readily habituate to this if handling is gentle and not associated with aversive experiences.

Behaviour tests are short lasting and tests that may be perceived negatively are balanced by tests that allow the chicks to explore and obtain food rewards.

Feed deprivation for a period of maximal 4 h, which occurs 3 times, is considered to cause small/moderate

discomfort.

7. Welke maatregelen heeft u getroffen om het ongerief tot een minimum te beperken?

Anesthesie:

- A A. Niet toegepast (geen aanleiding).
- B A. Niet toegepast (geen aanleiding).
- C A. Niet toegepast (geen aanleiding).
- D A. Niet toegepast (geen aanleiding).
- E A. Niet toegepast (geen aanleiding).

Pijnbestrijding:

- A A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- B A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- C A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- D A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.
- E A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.

To reduce the stress of handling, the chickens will be handled in a calm assured and gentle fashion. The durations of behaviour tests are short (maximum of 10 minutes) and will be shortened further where possible.

If hens suffer poor health during the study these will be taken out of the study and euthanized. Poor health implies, for example, a feather score of 3 or 4 (on a scale of 0 -4 , Bright A, TA Jones and MS Dawkins 2006 Animal Welfare 15:113-118:), or a score of 2 for longer than 2 weeks. Several days of significant poor body condition and general weakness (behavioural apathy) is also a reason for removing hens from the study. In this, the care takers have the lead in daily checking the hens' health and indicating when hens are to be euthanized.

8. Toestand van dieren na einde van de proef:

- A Het dier is na de proef in leven gelaten.
- B Het dier is na de proef in leven gelaten.
- C Het dier is na de proef in leven gelaten.
- D Het dier is na de proef in leven gelaten.
- E Het dier is na de proef in leven gelaten.

Toelichting:

Immediately after hatching male chickens not used in the experiment will be handed over to the research facilities. Furthermore, leftover female chickens will be handed over to the experimental facilities. After experimentation the used hens will also be handed over to the research facilities. The latter implies that hens may be maintained at [REDACTED] for production purposes or passed on to private people to live on as a companion animal, but if such is not feasible the animals are euthanized.

9. Welke alternatieven (vervanging, verfijning, vermindering) zijn voor de beschreven experimenten overwogen en waarom zijn deze verworpen?

Replacement:

Replacement is not possible, because this research focuses on the behaviour of laying hens. In vitro models etc. are not available for this type of experiments.

Refinement

Ill or injured animals will be removed from the test. Enrichment will be administered using wood shavings in spacious pens (4 chicks / 2 m²). The temperature will be adjusted to the needs of the chickens during development.

Reduction:

By integrating several research questions in one experiment relatively few animals are needed.

10. Namen van direct betrokkenen bij de dierproef (artikel 9- en 12-functionarissen):



Tabel registratiecode opties voor aanvraag 2012071.c (K14):

	1	2	3	4	5	6	7	8	9	10	11	12	13
					35	2	1	01					
A	1	51	5	12					01	1	1	2	3
B	1	51	5	12					01	1	1	2	3
C	1	51	5	12					01	1	1	2	3
D	1	51	5	12					01	1	1	2	3
E	1	51	5	6					01	1	1	2	3

Uw aanvraag 2012071.a, door u aangemeld vanuit DRS heeft van de DEC de status: 'Wijzigen' gekregen.

De DEC is van mening dat het doel van de proef opweegt tegen het te verwachten gering/ matige ongerief dat de dieren ondergaan. Voorafgaand aan een definitief advies heeft de DEC de volgende vragen en opmerkingen:

De DEC verzoekt u de proef wetenschappelijk te laten toetsen door derden, aangezien toetsing door de betrokken onderzoekers zelf niet aan de formele eisen voldoet.

Daarnaast verzoekt de DEC u bij 1.a. (te beantwoorden vraag) expliciet de hypothese te formuleren, die aan de proef ten grondslag ligt, mede met het oog op de relevante parameters voor het berekenen van het aantal benodigde proefdieren bij 4.c. (toelichting aantal dieren).

Bovendien verzoekt de DEC u bij 4.c. aantal dieren uitvoeriger te onderbouwen. U geeft aan, dat "Literature shows that a sample size of about 12 chickens per treatment group is sufficient", maar dit zegt niets over de vraag, of het niet met minder dieren kan.

U geeft bij 6.a. (proefschema) aan dat kuikens van alle behandelingen samen in een kooi worden gezet, terwijl bekend is dat één kip met afwijkend gedrag de hele koppel kan beïnvloeden. De DEC vraagt zich af, of dit de onderzoeksresultaten niet kan verstoren, aangezien u met name geïnteresseerd bent in gedrag en zij verzoekt u hierop in te gaan.

Tevens adviseert de DEC u, ten behoeve van de lichtinstelling bij de controlegroep te meten, hoeveel licht in de praktijk voorkomt in broedmachines (continu of afwisselend), aangezien niet uit te sluiten is, dat er licht naar binnen komt. Daarnaast verzoekt de DEC u de keuze voor 100-1000 lux te onderbouwen en duidelijker te beschrijven, hoe het licht wordt geregeld en welk soort licht, welk spectrum wordt gebruikt.

Tenslotte heeft de DEC nog enkele redactionele opmerkingen:

De DEC verzoekt u bij 4.c. de formulering "The number of experimental units is relatively small" weg te laten, aangezien het kleinst mogelijke aantal dieren de norm is.

Bovendien verzoekt zij u bij 6.a. een tijdschema te vermelden, waarin wordt aangegeven, wanneer welke testen worden gedaan, op welke leeftijd en hoeveel tijd er tussen de testen zit.

Tevens verzoekt zij u bij 6.a. (sound preference) aan te geven wat wordt bedoeld met "black either white noise".

Daarnaast verzoekt ze u bij 6.c. (bronnen van ongerief) aan te geven, dat er herhaaldelijk "feed deprivation" wordt toegepast.

Bovendien verzoekt de DEC u bij 7. (maatregelen ter beperking van ongerief) duidelijker te omschrijven, wat u onder "poor health" verstaat en bij 8. (toestand dieren na einde van de proef) duidelijker aan te geven, wat er met de dieren gebeurt na de proef, aangezien "handed over to the research facilities" erg vaag is.

Tot slot verzoekt zij u bij 9. (alternatieven) de tekst onder 'reduction' te wijzigen, aangezien de keuze voor hennetjes niet wordt ingegeven door de noodzaak tot vermindering, maar door het doel van de proef.

Na aanpassing zal de proef door de secretaris van de DEC worden afgehandeld in overleg met de proefdierdeskundige.

Uw aanvraag 2012071.b, door u aangemeld vanuit DRS heeft van de Secretaris DEC de status: 'Wijzigen' gekregen.

Indien de status op 'wijzigen' is gezet en u wilt deze aanvraag gaan wijzigen, dan selecteert u deze aanvraag en kiest u vanuit het menu 'bewerken aanvraag', en dan de optie 'wijzigen'. Er wordt dan een kopie van de originele aanvraag gemaakt. Deze kopie kunt u vervolgens wijzigen, en opnieuw aanmelden.

Met vriendelijke groet,


Secretaris DEC

Uw aanvraag 2012071.c, door u aangemeld vanuit DRS heeft van de Secretaris DEC de status: 'Positief advies na behandeling DEC' gekregen.

De DEC is van mening dat het doel van de proef opweegt tegen het te verwachten gering/ matige ongerief dat de dieren ondergaan en dat de vraag m.b.t. alternatieven voldoende is beantwoord.

Met vriendelijke groet,



Secretaris DEC