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Hens vary their vocal repertoire and structure when anticipating different types of reward

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27 **Abstract**

28 The vocalizations of nonhuman animals are considered potential indicators of motivational or
29 internal state. In many species, different call types, and structural variation within call types,
30 encode information about physical characteristics such as age or sex, or about variable traits
31 such as motivation. Domestic chickens, *Gallus gallus*, have an elaborate vocal repertoire,
32 enabling investigation into whether reward-related arousal is encoded within their call type
33 and structure. Twelve hens were given a Pavlovian conditioning paradigm using sound cues
34 to signal the availability of two food rewards (mealworms, normal food), one nonfood reward
35 (a container of substrate suitable for dustbathing), and a sound-neutral event, (sound cue, no
36 reward). A muted-neutral treatment (no sound cue, no reward) provided a baseline for vocal
37 behaviour. Sound cues preceded a 15 s anticipation period during which vocalizations were
38 recorded. Hens produced a 'Food call' (previously defined in other studies) in anticipation of
39 all rewards, including the nonfood reward. 'Food calls' and 'Fast clucks' were more prevalent
40 in anticipation of rewards, and most prevalent following the cue signalling the dustbathing
41 substrate, suggesting that this reward induced the most arousal in hens. The peak frequency of
42 'Food calls' made in anticipation of the dustbathing substrate was significantly lower than
43 those made in anticipation of food rewards, potentially reflecting differences in arousal.
44 Vocalizations that reliably indicate hens' motivational state could be used as measures of
45 welfare in on-farm assessment situations. Our study is the first to reveal variation in the
46 frequency-related parameters of the 'Food call' in different contexts, and to show prevalence
47 of different call types in reward and nonreward contexts, which may have implications for
48 welfare assessments.

Keywords: laying hens, motivation, referential, rewards, vocalizations.

50 Animal vocalizations contain information encoded within parameters such as frequency
51 (Townsend, Charlton, & Manser, 2014), amplitude (Gustison & Townsend, 2015; Reichard
52 & Anderson, 2015), rate of production (Clay, Smith, & Blumstein, 2012) duration
53 (Dentressangle, Aubin, & Mathevon, 2012) and energy distribution (Linhart, Ratcliffe, Reby,
54 & Špinka, 2015). Some information may be static, relating to age, sex or body size (Briefer &
55 McElligott, 2011; Charlton, Zhihe, & Snyder, 2009). However, variation in these acoustic
56 parameters may also provide ‘markers’ of internal states (Manteuffel, Puppe, & Schon, 2004;
57 Tallet et al., 2013). Internal states, in this context, refer to states of arousal induced by both
58 internal (e.g. hunger) and external (environmental) stimuli and the interactions between them.
59 Such states exist on a continuum, with arousal levels being in constant flux according to
60 changes in stimuli and internal adjustments to these stimuli (for a detailed discussion see
61 Berridge (2004)).

62
63 Flexible traits, such as signaller motivation, can be reflected in vocal frequency, amplitude,
64 duration and rate in both humans (Scherer, 1986) and animals (Briefer, 2012; Taylor & Reby,
65 2010), as shown in meerkats, *Suricata suricatta* (Hollén & Manser, 2007) and rats, *Rattus*
66 *norvegicus* (Knutson, Burgdorf, & Panksepp, 2002). The flexible features of vocalizations
67 tend to be subject to certain ‘motivation-structural rules’ (Morton, 1977). According to this
68 concept, vocalizations produced in one motivational context (e.g. a hostile situation) should
69 vary in structure from vocalizations produced in a very different motivational context (e.g.
70 friendly interactions; Morton, 1977). This theory has been tested in many species including
71 domestic dogs, *Canis lupus familiaris* (Yin & McCowan, 2004), chimpanzees, *Pan*
72 *trogodytes* (Siebert & Parr, 2003), coatis, *Nasua nasua* (Compton, Clarke, Seidensticker, &
73 Ingrisano, 2001) and elk, *Cervus canadensis* (Feighny, Williamson, & Clarke, 2006). For
74 most of these animals, the call types commonly produced in hostile contexts are long in
75 duration with a low frequency, whereas in fearful or nonaggressive contexts, short, high-

76 frequency, tonal calls are produced (see review by Briefer, 2012). In addition to different call
77 types, motivation may also be encoded within a sound's structure. For example, the frequency
78 of particular sounds may be lowered when a signaller is feeling aggressive (Bee & Perrill,
79 1996).

80

81 Certain animal vocalizations function referentially, passing information about specific
82 environmental stimuli to receivers (Macedonia & Evans, 1993). To fulfil the criteria for
83 functional reference, calls must be elicited by a narrow range of stimuli, and evoke a response
84 in the receivers as if they had experienced the stimuli themselves (Evans, 1997; Macedonia &
85 Evans, 1993; Marler, Evans, & Hauser, 1992). For example, some species of primate produce
86 different call types according to particular threats or predators (Murphy, Lea, & Zuberbühler,
87 2013; Zuberbuhler, Noe, & Seyfarth, 1997), which elicit distinctive adaptive responses in the
88 receivers. Functionally referential vocalizations may also encode motivational information at
89 the same time as being referential (Hollén & Manser, 2007). In these instances, the call rate or
90 structural variation within call types may encode information relating to arousal, motivation
91 or urgency (Clay et al., 2012; Manser, 2001). Townsend and Manser (2013) described a
92 motivational-referential continuum, giving the example of meerkats which produce calls that
93 refer to nearby predators and also deliver information about the urgency of the threat. The
94 'Food call' of the chicken, *Gallus gallus* (Collias, 1987; Evans & Marler, 1994) has been
95 described as the most rigorous example of food-specific functional reference within terrestrial
96 animals (Clay et al., 2012). This call appears to meet all the criteria of functional reference;
97 there is acoustic specificity between the stimulus (food) and the signal, and playback elicits
98 feeding-related behaviours in receivers (Clay et al., 2012; Townsend & Manser, 2013). It is
99 also likely that the 'Food call' contains motivational information in its acoustic variables.

100

101 The anticipation of rewards is thought to increase arousal in animals induced by changes in
102 motivational state, and this, in turn, may elicit vocalizations. Rats, for example, produce
103 ultrasonic vocalizations at 50 kHz in anticipation of rewards such as the presence of a play
104 partner, during tickling from a familiar human or in response to a cue signalling food
105 (Burgdorf & Panksepp, 2006; Knutson, Burgdorf, & Panksepp, 1998; Panksepp & Burgdorf,
106 2000). By contrast, negative stimuli such as the presence of a predator, or a cue signalling an
107 electric shock, tend to elicit a 22 kHz vocalization in rats (Knutson et al., 2002). This
108 knowledge, theoretically, allows us to assess whether a rat is in a 'rewarding' environment,
109 which has implications when determining their welfare. Clear indicators about whether
110 animals are experiencing rewarding or nonrewarding environments may guide decisions made
111 within management systems. The anticipation of rewards induced by signals has been linked
112 to 'pleasure-based' (dopaminergic) activity in the brain (Berridge, 1996). Therefore, while
113 stressful environments are known to have deleterious effects on productivity (Broom, 1991),
114 it is reasonable to assume that the reduction of stress through the provision of rewarding
115 environments could positively influence animal health and productivity (Boissy et al., 2007).

116

117 The domestic chicken is a good candidate for the study of vocalizations made in anticipation
118 of rewards for three main reasons. First, the behaviour of chickens in anticipation of rewards
119 has already been well documented (Garland, Castellote, & Berchok, 2015; Moe et al., 2014;
120 Moe, Nordgreen, Janczak, Spruijt, & Bakken, 2013; Moe et al., 2009; Zimmerman, Buijs,
121 Bolhuis, & Keeling, 2011). Second, behavioural data suggest that the motivational state of
122 chickens changes according to the type of reward (Garland et al., 2015; McGrath, Burman,
123 Dwyer, & Phillips, 2016), and therefore it may be possible to link anticipatory behaviour with
124 vocalizations produced in anticipation of rewards. Third, the chicken has a wide and varied
125 vocal repertoire. Between 20 and 25 discrete calls have been documented in various studies
126 (Collias & Joos, 1953; Evans, 1993; Evans & Evans, 1999; Kruijt, 1964; Marx, Leppelt, &

127 Ellendorff, 2001; Woodgush, 1971) including those classed as referential (Evans & Evans,
128 2007). Interestingly, it seems that motivational information may be encoded within referential
129 calls. Alarm calls, for example, differentiate between terrestrial threats and aerial predators
130 (Evans, Evans, & Marler, 1993) while simultaneously encoding the motivational state of the
131 bird (Kokolakis, Smith, & Evans, 2010).

132

133 Chickens have been shown to modify the rate and numbers of their 'Food calls' in response to
134 different types of food reward (Marler, Dufty, & Pickert, 1986; Wauters, Richard-Yris, Pierre,
135 Lunel, & Richard, 1999). These changes appear to reflect variation in motivational state
136 according to food type, which indicates there is an opportunity to test for motivational
137 information within a referential call. The 'Food call' of chickens is described by various
138 authors as having a characteristic appearance consisting of trains of pulsatile calls delivered in
139 a regular temporal pattern, emphasizing low frequencies, and at a rate of 4–10 per s (Collias,
140 1987; Evans & Marler, 1994). They are given by both males and females, and their structure
141 facilitates location of the sender (Hughes, Hughes, & Covalt-Dunning, 1982). No studies have
142 investigated vocalizations produced by chickens in anticipation of other types of reward, or
143 variation in their acoustic parameters. Therefore, the goal of this study was to characterise
144 vocalizations made in anticipation of different types of reward (different food types known to
145 be 'rewarding' to chickens, Bruce, Prescott, & Wathes, 2003, and a substrate suitable for
146 dustbathing). We aimed to investigate whether call parameters varied within the call types
147 according to reward type, and according to whether it was a reward or nonreward. To achieve
148 this, we experimentally induced anticipation of these rewards, using a Pavlovian conditioning
149 paradigm. We tested the hypothesis that chickens would produce specific call types in
150 anticipation of different rewards. We also tested the hypothesis that vocal parameters would
151 differ according to the perceived quality of the reward. These differences would provide

152 information on the motivational state of the signaller, and therefore could be used as
153 indicators of baseline welfare.

154 **Methods**

155 **Subjects and Housing**

156 Twelve ISA Brown hens, approximately 18 weeks old, were obtained from the University of
157 Queensland's poultry unit. The hens were housed in groups of three in pens measuring 266 x
158 266 cm and 133 cm high. The floor of the home pen was shredded rubber chip, and each pen
159 contained a metal structure used as a perch (149 x 119 cm and 41 cm high) and two nestboxes
160 (40 x 35 cm and 45 cm high). Food (standard layer pellets) and water were available ad
161 libitum in the home pens. The housing had natural as well as artificial light (the latter on
162 between 0600 and 1800 hours). There was no temperature control, but all experimental work
163 was conducted between 0800 and 1230 hours to standardize the conditions. Hens were
164 individually identifiable to the experimenter (N.M.) by plumage colouring, marking and comb
165 size, avoiding the need for individual marking or ringing.

166

167 **Ethical note**

168 The methods used in this study were approved by the University of Queensland Animal
169 Ethics Committee (Ref. SVS/314/12). The experiment used a minimum number of hens based
170 on related published studies. The ISA Brown hen is a commonly used strain in production
171 environments, and therefore conclusions about welfare would have a general application.
172 Hens were not marked and the experimenter used natural markings on the hens for individual
173 recognition. The procedures used in this experiment could have exposed hens to stress
174 through handling when moving from the home pen to the experimental pen, and when
175 isolated in the experimental pen. The experimenter was trained in handling chickens, and
176 therefore any such stress was minimized, and the experimental procedure was, by its nature,

177 mainly rewarding, with no aversive conditions used. Cleaning was performed routinely every
178 few days. Hens habituated quickly to these procedures. They were housed in large pens, each
179 furnished with a perch, a tray of dustbathing substrate and two nestboxes to cater for natural
180 behaviour. In addition, they could access a cauliflower or broccoli hanging from a piece of
181 string above the ground as food enrichment.

182

183 **Treatment Groups**

184 Hens were subjected to a Pavlovian conditioning paradigm as used by Zimmerman et al.
185 (2011) and Moe et al. (2009). In our experiment, an initially neutral stimulus (conditioned
186 stimulus, CS) was repeatedly paired with the presentation of one of three different rewards
187 (mealworm, normal food or dustbathing substrate) or a sound-neutral event (an empty
188 compartment) which served as the unconditioned stimuli (US). We used different sound cues
189 for the conditioned stimuli all 5 s long: ‘ring’ (ringing of a rotary dial telephone), ‘beep’ (an
190 alarm-clock style beep), ‘buzz’ (a buzz sound as in a game show) and ‘horn’ (an old-
191 fashioned car horn sound). A muted neutral (MN) treatment (5 s of ‘nothing’, no CS or US)
192 was used to control for the effect of sound in the other treatments. The sound cues were
193 played from a computer at a sound pressure level of 75 dBA, measured 1 m from the centre of
194 the experimental chamber. Each of the four sound cues was used to signal the presence of
195 each type of US. Consequently, four cue groups of three hens (from the same home pen) each
196 experienced different combinations of CS and US (Table 1).

197 **Experimental Apparatus**

198 An experimental pen (200 x 125 cm and 60 cm high) was located in a sound-proofed room
199 adjacent to the room in which the hens were housed. The pen contained two compartments of
200 equal size, a waiting compartment and a reward compartment, separated by a wire-mesh
201 partition and connected by a swing door in the middle of the partition (Fig. 1). The door could
202 be locked and released by increasing or decreasing an electrical current passing through an

203 electromagnet attached to it, and only opened in the direction of the reward compartment.
204 Three of the four walls of the experimental pen were made of plywood and one was made of
205 wire-mesh to allow video recordings of both compartments. A lamp, secured to the middle of
206 the outer wall at 60 cm from the floor, could be operated by the experimenter who sat behind
207 a screen out of sight of the hens during tests. This light shone into the reward compartment
208 and was used to highlight the reward and indicate that the door was open.

209

210 The apparatus used for rewards comprised a white food bowl, a tray filled with topsoil/sand
211 mix and the hens' normal feeders, enabling hens to discriminate between reward types. The
212 topsoil/sand mix was chosen after a review of the literature on functional substrates for
213 dustbathing, and its dry crumbly texture suggested that it was suitable for this purpose
214 (Olsson & Keeling, 2005). Duplicates of the white food bowl containing food and a white tray
215 filled with the topsoil/sand mix were put in the home pens 3 days before training started to
216 allow hens to become accustomed to them.

217

218 **Training Procedure**

219 The training consisted of several phases similar to those used by Zimmerman et al. (2011)
220 with adjustments in the length of each phase due to the number of conditioned stimuli used.
221 As a result of the hens learning to enter the reward compartment more slowly, training took
222 place over 25 days (compared with 22 days in Zimmerman et al. 2011). In phase 1 (Days 1 -
223 7) hens were trained to use the swing door. This initial training was done in groups of three to
224 increase the speed of training. During the first 2 days the door was kept fully open, and a trail
225 of sunflower seeds led through the door into the reward compartment where the white bowl
226 held more sunflower seeds. Each home pen group of three hens was allowed to accustom
227 themselves to moving from the waiting compartment to the reward compartment following
228 the trail of seeds. On the following 5 days, home pen groups were trained to go through the

229 door, the opening width of which was gradually reduced more on each day. During this
230 period, the hens were food deprived for an average of 2 h and mealworms were placed in the
231 white bowl in the reward compartment to motivate them to go through the door. Each group
232 of three hens experienced eight consecutive trials. On the final day, the door was fully closed,
233 but unlocked, so that the hens had to push through it to gain access to the mealworms.

234

235 In phase 2 (Days 8–13), the hens were individually trained to recognize the specific CS for
236 each of the rewards. The containers containing the dustbathing substrate were removed from
237 the home pens from this time onwards. In this phase, the door was kept unlocked and hens
238 were given 10 min to go through the swing door after their particular CS for mealworm,
239 dustbathing substrate or normal food was played and the light switched on. All hens entered
240 the compartment within the 10 min time limit. After consumption of the reward, the light was
241 switched off and the hens were guided back into the waiting area by the experimenter. Each
242 cue group was trained for one stimulus on one day and each individual experienced three
243 consecutive trials. Hence, during the 6 days, each hen experienced six trials of each stimulus
244 with the door unlocked. In phase 3 (Days 14–17), the swing door was locked, and the CS and
245 light signal were made contingent on the behaviour of the hen. Hens were placed individually
246 into the waiting compartment and allowed to try to push through the locked door twice before
247 the CS was played, the light was switched on and, simultaneously, the door was unlocked
248 (CS/Light/Door). This procedure was repeated three times and then the CS /Light/Door
249 sequence was performed at random when the hen was not near the door. When the hen went
250 through the door immediately after the CS/light was given in five consecutive trials, the
251 training session was ended and hens were returned to their home pen. In phase 4 (Days 18–
252 19), a trace conditioning procedure (Moe et al., 2009) was used to accustom the hens to an
253 interval between the CS being played and activating the light signalling the door was
254 unlocked. The CS was played for 5 s and the interval between the end of the CS and the light

255 signal was gradually increased from 0 to 15 s over five consecutive trials for each individual
256 hen on each day. Hens were deemed to have successfully reached our criterion when they
257 went through the swing door within 5 s after the light had been switched on. In phase 5 (Days
258 20–22), all hens were introduced to their sound-neutral CS. The sound-neutral CS was not
259 introduced in an earlier phase to aid consolidation of learning with regard to going through
260 the door after the trace delay. In these sound-neutral trials, nothing happened after the light
261 had been switched on. The light was kept on for 15 s and then switched off. In phase 6 (Days
262 23–25), rewarded (mealworm, dustbathing substrate, normal food) and sound-neutral trials
263 were presented in a randomized order, with each cue being presented at least once to each hen
264 on each day. Hens successfully reached our criterion when they went through the swing door
265 within 5 s on every rewarded trial. Hens learned at different speeds during this training phase;
266 however, there were no consistently ‘slow learners’ for all phases, and training schedules
267 were adjusted to ensure that all hens progressed to the next stages together, so that there was
268 no bias in exposure to stimuli for individual hens.

269

270 **Test Procedure**

271 For testing, a hen was collected from her home pen and put in the experimental pen. The
272 order of testing was determined using an orthogonal Latin square design in which every single
273 condition followed another on two occasions. Each hen received one test session per day on 5
274 consecutive days. Hens were deprived of food for an average of 1.5 h prior to testing, and
275 deprived of a substrate suitable for foraging and dustbathing in their home pens for all 5 days
276 of the test period. Each test session consisted of presentation of each of five stimuli: three
277 reward treatments (mealworm, dustbathing substrate, normal food), one sound-neutral and
278 one muted-neutral trial. At the start of each session a hen was allowed to habituate to the
279 experimental pen for 30 s. Then the appropriate CS was given for 5 s, after which behaviour
280 and vocalizations were recorded for 15 s before the light was switched on signalling the door

281 was unlocked. There was no CS in the muted-neutral trial, but behaviour was recorded for 15
282 s from when the trial started. In the mealworm trial, after the CS and the 15 s anticipation
283 period, the door was unlocked and the hen entered the reward compartment and ate the
284 mealworm. Then the light was switched off and the hen was ushered gently into the waiting
285 compartment by the experimenter who held the swing-door open. In the normal food trial, the
286 same happened except that the hens were allowed 1 min to feed before the light was switched
287 off and the hen was returned to the waiting compartment. In the dustbathing substrate trial,
288 the same process was followed except that the hens were allowed to dustbathe or forage (with
289 no food present) for 5 min before the light was switched off and the hen was returned to the
290 waiting compartment. If the hens stopped feeding or foraging/dustbathing and walked away
291 from the stimulus, or engaged in other behaviour in other parts of the pen for a continuous
292 period of 10 s, then the trial was ended. In a sound-neutral trial, the CS was given and, after a
293 15 s anticipation period, the light was switched on but the door did not open. In a muted-
294 neutral trial, no sound cue was given, and behaviour and vocalizations were recorded for 15 s.
295 In all trials, between the end of the trial and the start of a new waiting period, there was an
296 intertrial interval of 10, 20 or 30 s (balanced between hens), to prevent hens from anticipating
297 the start of the next trial,

298
299 During the test procedure, vocalizations were recorded using a Sennheiser ME66 condenser
300 shotgun microphone connected to a Tascam DR100 MkII DAT recorder. Gain settings were
301 set to High and the rotary dial input gain setting was set at 6. Recordings were made with 24
302 bit resolution at a sampling rate of 44.1 kHz. The DAT files were transferred to a PC (Dell) to
303 analyse the vocalizations using Raven Pro: Interactive Sound Analysis Software (Version 1.5,
304 Cornell Laboratory of Ornithology, Ithaca, NY, U.S.A.).

305

306 **Acoustic analysis**

307 Spectrograms of recordings were generated using Raven Pro 1.5 (Cornell Laboratory of
308 Ornithology; 1792-sample Hann window, 35.4 Hz filter bandwidth, 5.38 Hz frequency
309 resolution (grid spacing), discrete Fourier transform (DFT) size of 8192 samples and time grid
310 hop size 200 samples (88.8% overlap)). Measurements were made in Raven Pro and values
311 were imported into Excel by syllable. A syllable is defined as a sound that makes a
312 continuous impression in time on the spectrogram. A call is defined as a clearly discernible
313 sequence (both audibly and visually) of syllables grouped together. Recordings with
314 extensive background noise ($N=94$) were excluded from further analysis (Fig. 2). The
315 difference in percentage of calls rejected by treatment was not significant ($\chi^2_8=0.26$, $P>0.05$).
316 No vocalizations were elicited in 23 of the 300 treatments, and this number was distributed
317 across contexts as follows: dustbathing substrate: 5; mealworm: 6; normal food: 2; sound-
318 neutral:3; muted-neutral: 7.

319

320 **Call Classification**

321 The call classification process followed those used in previous studies, including Garland et
322 al. (2015) and Rekdahl, Dunlop, Noad, and Goldizen (2013). There are no fully automated
323 classification procedures that do not include a qualitative component. Manual classification is
324 commonly used as a starting point as this method picks up the subtle features of the sounds
325 that quantitative measures will miss. However, because of these subtle differences, there is a
326 need to test the robustness of a manual classification. In this study, we first manually
327 classified the sounds and then carried out a classification and regression tree analysis (CART)
328 and Random Forest analysis to corroborate this initial classification (see below). A high
329 percentage agreement between the manual and quantitative methods suggests that the manual
330 classification consistently and correctly groups the sounds into types, and therefore is robust
331 and repeatable. A total of 546 calls were manually classified. The data were then grouped by

332 call in Excel and calculations were made for the temporal variables. Frequency variables were
333 averaged across calls. All variables measured are detailed in Table 2.

334

335 Descriptive analyses were performed on call types (Appendix Table A1). Example
336 spectrograms of these call types were visually compared with already documented chicken
337 calls (Collias, 1987; Evans and Evans, 2007).

338

339 Hens have distinct call types within their repertoire, but some calls also blend from one type
340 into another on a continuum of calls. Therefore, we created a 'Mixed' call category to include
341 these calls. The 'Food call' given by chickens was identified by comparing our recordings
342 with published spectrograms (Evans & Evans, 2007; Sherry, 1977). These calls were
343 characterized by a consistent fast rhythm and low pitch (Table A2). Of a total of 60 'Food
344 calls', 24 were made by one hen. To reduce bias, 16 of this hen's food calls (randomly
345 chosen) were removed from the analysis to better balance the number of calls made by
346 individuals.

347

348 To validate the manual classification of calls, we used a nonparametric CART and Random
349 Forest analysis. Applying the CART method, a decision tree was constructed using the rpart
350 package in R, which does not require independence of samples. In a decision tree a set of
351 hierarchical decision rules is created. Each rule can branch into another rule or a terminal
352 category. At each step, the split is made based on the independent variable that results in the
353 largest possible reduction in heterogeneity of the dependent variable; this is called the 'Gini
354 index', which measures impurity or 'goodness of split' (Breiman, Friedman, Stone, & Olshen,
355 1984). The optimal decision tree must contain criteria that classify the data as accurately as
356 possible, without 'overfitting' the data. Therefore, we pruned the tree to minimize
357 misclassification rate, by choosing a complexity parameter that minimizes the cross-validated

358 error. We also conducted a Random Forest analysis using the randomForest package in R
359 (Liaw & Wiener, 2002). This classification tool randomly selects a subset of predictor
360 variables and creates a 'forest', or a collection of decision trees (Breiman, 2001). The
361 aggregation of trees evaluates the classification uncertainty of each tree [the out of bag
362 (OOB) error] in addition to ranking the importance of each predictor variable (i.e. the call
363 variables we measured; Breiman, 2001). As Random Forests estimate error internally, no
364 cross-validation is required (Breiman, 2001). Based on the lowest OOB error, the number of
365 predictors randomly selected at a node for splitting was set to three and 1000 trees were
366 grown. Classification success was evaluated using the overall OOB error rate and individual
367 call type errors.

368

369 **Statistical analyses of call types**

370

371 All analyses of call types were performed in R (R CoreTeam, 2013). The prevalence of call
372 types used by hens was calculated by treatment. Prevalence of call types was analysed using a
373 generalized linear mixed model (GLMM) within the lme4 package (Bates et al., 2015) with
374 'treatment' as the predictor variable and each call type as the response variable with subject
375 ID as the random effect. A mixed model was used to account for the within-bird variance
376 associated with repeated measurements. A negative binomial model was used as the most
377 appropriate method to analyse these data, as these models allow for overdispersed data.

378 Incident rate ratios and 95% confidence levels were extracted, and mean predictions were also
379 checked against observed data to ensure they did not deviate, as deviation would indicate a
380 poorly fitting model. We used the function glmmADMB to run all negative binomial models.

381

382 To determine whether hens varied their call structure in anticipation of different rewards, we
383 analysed variation of acoustic parameters within call types. Only the call types 'Single

384 clucks', 'Double clucks', 'Fast clucks', 'Food calls', 'Whines' and 'Gakel calls' were used in
385 this analysis. 'Singing' calls ($N=16$) were eliminated due to the small number of calls.
386 'Mixed' calls ($N=124$) were also excluded as their structure was highly variable and therefore
387 they were difficult to classify. Many of the acoustic variables were highly correlated.
388 Therefore, we conducted a factor analysis (FA) with varimax rotation using the function
389 'factanal'. Acoustic variables were log transformed to better match hearing perception in
390 vertebrates (Cardoso, 2013), and plots were examined for linear relationships between
391 variables. The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy (MSA) was used
392 to distinguish which variables should be included in the FA. This measure indicates whether a
393 variable belongs to a family of variables psychometrically (Dziuban & Shirkey, 1974).
394 Variables with MSA scores below 0.5 indicate that an item does not belong to a group and
395 may be removed from the FA. Following these criteria, the variable 'Number of syllables'
396 (MSA score of 0.35) was removed from the FA.
397
398 In a preliminary FA, the model did not converge because variables 'Centre Frequency', 'Q1
399 frequency' and 'Q3 frequency' had very low levels of uniqueness (less than 0.0000001).
400 These variables were removed, and the models were run on the remaining variables, moving
401 from a one-factor up to a four-factor model. 'Call duration' and 'Average Syllable Length'
402 loaded on to separate individual factors and were therefore excluded from the FA and tested
403 as separate responses. The overall KMO test output of 0.69 and Bartlett's test of sphericity
404 ($P<0.001$) indicated that the FA was justified (McGregor, 1992). Using the package
405 nFactors(), the optimal number of factors as determined by both eigenvalues and parallel
406 analysis was two. 'Bandwidth 90%', 'Delta Frequency' and 'IQR Bandwidth' loaded on to
407 Factor 1, with 'Bandwidth 90%' loading the highest (0.98), and 'Peak', 'Low' and 'High
408 Frequency' loaded on to Factor 2, with 'Peak Frequency' loading the highest (0.96). To
409 facilitate interpretation of results, the variables that loaded highest on to each factor,

410 'Bandwidth 90%' (from Factor 1) and 'Peak Frequency' (from Factor 2), were used for the
411 subsequent call structure analysis. 'Call Duration' and 'Average Syllable Length' were also
412 analysed to detect whether hens altered the temporal structure of their calls in anticipation of
413 different rewards.

414

415 Variation in acoustic parameters for each call type was analysed using linear mixed-effects
416 models (LMMs) from within the lme4 package (Bates et al., 2015). The assumptions of
417 normal distribution, linearity and homoscedasticity of the residuals were checked by visual
418 inspection of residual plots and by Shapiro–Wilks tests. We computed parameter estimates
419 using the maximum likelihood method, and the significance of predictor variables was tested
420 using maximum likelihood ratio tests (anova() function in R). Each response variable was
421 modelled separately. For all LMMs the chi-square statistic (χ^2), degrees of freedom and
422 associated *P* values are reported. (Full results are available in Appendix Table A3.) Post hoc
423 analyses were conducted using the lsmeans package (version 2.20-23) in R, applying the
424 Tukey method for individual comparisons of different factor levels within the same model,
425 incorporating an adjustment of *P* values for multiple comparisons. A Bonferroni correction
426 was applied to account for the number of variables being tested using the same data set, and
427 therefore *P* values of and below 0.01 were considered significant.

428

429 Model selection was carried out using Akaike information criterion (AIC) scores, and by
430 performing chi-square tests to compare models using the anova() function, with values of
431 $P < 0.05$ considered significant (Appendix Table A4). Initial models included 'Preceding
432 Treatment' as a predictor variable to establish whether there were any carryover effects
433 between treatments. As there was no significant effect of preceding treatment, this predictor
434 was left out of the final models. Final models included the following sets of predictor
435 variables: (1) Treatment only, (2) Treatment and Cue Group, (3) Treatment, Cue Group and

436 Day. Model comparison and AIC scores are detailed in Appendix Table A4. Residual plots
437 were checked by using the plot() function to check for any patterns in the data. All final
438 model outputs are detailed in Appendix Tables A5–A10.

439

440 **Results**

441 **Call classification**

442 Hens produced eight different call types according to both the manual classification and
443 CART analysis, suggesting a high agreement between the two methods and a high probability
444 this call repertoire is ‘correct’. These ranged from the very short ‘Single cluck’, with a call
445 duration of 0.08 s, to a long ‘Gakel call’ (Zimmerman and Koene, 1998; 3.1 s). ‘Single
446 clucks’ and ‘Double clucks’ were short sharp clucks with either one or two syllables. Two
447 other ‘cluck’ type calls, the ‘Fast cluck’ and the ‘Food call’, were structurally similar to the
448 ‘Single cluck’ and ‘Double cluck’, but consisted of a longer series of short sharp clucks (Fig.
449 6). The ‘Fast cluck’ was not as consistent in rhythm or pitch as the ‘Food call’.

450

451 Hens also produced long, high-pitched calls such as a ‘Whine’ (Fig. 7), which was
452 characterized by a wide frequency range (delta frequency = 274 Hz), compared with the
453 ‘cluck’ calls whose frequency range was from 162.9 to 173.7 Hz (Table A2). ‘Gakel calls’
454 were also identifiable by having a harsh or whiny tone, and a high frequency range (246 Hz).
455 A small number ($N=16$) of calls identified as ‘Singing’ (Collias, 1987) also had a high pitch
456 (644.6 Hz) and frequency range (366.8 Hz). ‘Mixed’ calls could not be categorized into any
457 of the other groups due to their high structural variation.

458

459 All variables listed in Table 2 were available for construction of the CART decision tree, and
460 the variables CART selected in tree construction were ‘Call duration’, ‘Maximum frequency’,
461 ‘Maximum syllable length’, ‘Number of syllables’, ‘Peak frequency’ and ‘Average syllable

462 length'. The tree correctly classified over 69% of calls (Fig. 3). The first branch in the tree
463 was based on call duration, which separated the cluck call types ('Food call', 'Fast', 'Single'
464 and 'Double clucks') from the 'Gakel calls' and 'Whines'. Further branching was heavily
465 influenced on the longer calls by duration of the call and maximum frequency, and on the
466 shorter calls by the number of syllables and syllable length, as well as acoustic frequency
467 parameters. The analysis resulted in eight terminal nodes (call categories), with 'Fast Clucks'
468 being the most prevalent in two terminal nodes.

469

470 Random Forest correctly classified a high number of calls (OOB estimate error rate was
471 26.6%; Appendix Table A11). This represents a high level of agreement in classification with
472 our manual classification of calls. The most important variables used by Random Forest
473 classification were Call duration and Number of syllables (mean decrease in Gini index = 88
474 and 72, respectively), followed by Maximum syllable length and Average syllable length
475 (mean decrease in Gini index = 62 and 55, respectively; Appendix Table A12). The majority
476 of misclassifications occurred within the 'Singing' category which had a low number of
477 examples, and a high misclassification rate and this increased the measure of error. 'Fast
478 clucks', 'Food calls' and 'Mixed' calls also had a moderate misclassification rate when
479 compared with our manual classification. It was expected that 'Mixed' calls would be difficult
480 to classify quantitatively, and this was reflected in the misclassification rate. 'Mixed' calls
481 contained a number of calls that represented different call types blending into others; for
482 example, 'Food calls' may blend into a short 'Whine'. 'Food calls' and 'Fast clucks' have
483 very similar structures. However, the CART analysis correctly classified 80% of 'Food calls'.
484 Only calls that demonstrated the regular temporal and acoustic structure described previously,
485 and that were visibly similar to a spectrogram published by Evans and Evans (2007), were
486 selected as 'Food calls' and analysed further.

487

488 **Prevalence of call types**

489 The call repertoire of hens varied significantly depending on the type of reward. The
490 prevalence of 'Food calls' and 'Fast clucks' was significantly greater in anticipation of all
491 rewards compared to both neutral treatments (Fig. 4a, b) suggesting that these call types are
492 produced more when hens anticipate a positive event.

493

494 **Differences in call parameters**

495 As 'Food calls' were prevalent in reward contexts but scarce in neutral treatments (Fig. 4a),
496 call parameters were only compared in anticipation of rewards. Only the peak frequency of
497 'Food calls' varied in anticipation of rewards, where they were produced at significantly
498 lower peak frequencies in anticipation of the dustbathing substrate compared with the
499 mealworm reward (dustbathing substrate: 411.85 Hz; normal food: 467.55 Hz; mealworm:
500 487.16; $\chi^2_2=8.78$, $P=0.01$; Fig. 5).

501

502 The 90% Bandwidth of 'Fast clucks' was significantly lower during all three reward
503 treatments than during the muted-neutral treatment (sound-neutral: 86.71 Hz; normal food:
504 89.96 Hz; dustbathing substrate: 90.04 Hz; mealworm: 98.86; muted-neutral: 126.81 Hz;
505 $\chi^2_4=13.25$, $P=0.01$). Interestingly, 'Fast clucks' were also produced at lower peak frequencies
506 in anticipation of the dustbathing substrate and mealworm rewards, which were presumably
507 high-value rewards, than in the muted-neutral treatment, although the results were not
508 significant at $P\leq 0.01$ (dustbathing substrate: 402.66 Hz; mealworm: 403.12; normal food:
509 409.19 Hz; sound-neutral: 56.59 Hz; muted neutral: 548.59 Hz; $\chi^2_4=11.30$, $P=0.02$). The
510 above variations suggest that these call types encode information about motivational state
511 according to different types of reward. However, the frequency of 'Double clucks' and
512 'Single clucks' did not vary between treatments ($P>0.01$), which indicates that shorter calls
513 do not encode information about hens' internal states (see Appendix Tables A9 and A10). The

514 acoustic parameters of 'Whines' and 'Gakel calls' did not vary between treatments ($P>0.01$;
515 see Appendix Tables A7 and A8).

516

517 **Discussion**

518 The results of this study show that hens produce 'Food calls', 'Fast clucks', 'Double clucks'
519 and 'Single clucks' when anticipating rewards. Importantly, hens produced 'Food calls' when
520 anticipating a dustbathing substrate, which contradicts the suggestion that 'Food calls' made
521 by chickens are functionally referential (Clay et al., 2012; Evans & Evans, 1999). The peak
522 frequency of 'Food calls' made in anticipation of the dustbathing substrate was 45–75 Hz
523 lower than those made in anticipation of food rewards, potentially reflecting differences in
524 arousal within the same behavioural state.

525

526 We found that all four call types produced by hens in anticipation of rewards ('Food calls',
527 'Fast clucks', 'Double clucks' and 'Single clucks') are structurally similar calls, with a
528 fundamental frequency of around 400 Hz and a distinct first harmonic. Calls were mainly
529 differentiated by the number of syllables, with 'Food calls' being a series of 3–15 syllables,
530 produced at a slightly higher peak frequency than 'Fast clucks'. During the neutral treatments
531 (i.e. no rewards), hens produced very different signals. These were long, high-frequency,
532 tonal 'Whines' and long, distinctive, harsh-sounding 'Gakel calls'. 'Whines' produced during
533 our experiment were very similar to the 'whines of frustration' given by red junglefowl, *G.*
534 *gallus*, in contexts when food is shown or offered, but subsequently not released to the birds
535 (Collias, 1987). Similarly, 'Gakel calls' are given by hens in frustrative nonrewarding
536 contexts (Garland et al., 2015; Zimmerman & Koene, 1998; Zimmerman, Koene, & van
537 Hooff, 2000). Hens also produced 'Mixed' calls, which did not have a regular or specific
538 structure. The anticipatory period in our experiment may have induced conflicting arousal
539 states; a drive to acquire the reward may have existed simultaneously with frustration induced

540 by not having instant access to the reward. The grading of calls, where one signal merges into
541 another, occurs in red junglefowl and domestic chickens, particularly under intermediate
542 stimulus conditions (Collias, 1987) or in situations of varying intensity (Konishi, 1963).
543 Therefore, it appears that hens' call types indicate the motivational state of the signaller;
544 short, sharp 'clucks' and 'Food calls' tend to be produced in anticipation of rewards
545 (indicating possible excitement) and longer, higher frequency 'Whines' and 'Gakel calls' in
546 frustrative non-rewarding contexts. The vocalizations of other animals have been linked to
547 levels of arousal, and in some cases, valence (Briefer, 2012). Therefore, these findings
548 present an opportunity to explore how hens' vocal communication could be used as markers
549 of their welfare in on-farm assessments.

550

551 An important finding of this study was that 'Food calls' were not solely produced in the
552 context of food, but also given in anticipation of both food and nonfood rewards. This result
553 contradicts claims that they are functionally referential (Clay et al., 2012; Evans & Evans,
554 1999). Other studies have indicated that food calling can occur in the absence of food, and
555 either may be associated with stimuli that, in the past, reliably predicted the presence of food
556 (Moffatt & Hogan, 1992; Wauters & Richard-Yris, 2002) or may be separation calls (Hughes
557 et al., 1982; Konishi, 1963). Certainly, there is evidence to suggest that the majority of 'Food
558 calls' made by other species are not food-specific, but rather food-associated, as they do not
559 fulfil the strict criteria for functional reference with regard to either production or perception
560 (for a review, see Clay et al., 2012). In these cases, 'Food calls' may function more as social
561 recruitment calls, to enhance defence, reproductive or social status, or alternatively as a
562 method to reduce competition by announcing resource ownership (Clay et al., 2012). Male
563 chicken 'Food calls' appear to be used to attract females by inducing orientation towards the
564 sound source (Evans & Evans, 1999). However, 'Food calls' made by females may function
565 to recruit others, either to share a resource or to ensure extra security (vigilance) when

566 engaged in foraging, feeding or dustbathing (Clay et al., 2012; Townsend, Zöttl, & Manser,
567 2011). Hence, information appears to be contained within the overall structure of hens' calls
568 (with hens producing short sharp cluck sounds when the signaller has located a resource).
569 However, there is no evidence in the literature to suggest that these calls differ between
570 various resources.

571

572 Many species of animal vary the structure of specific call types depending on their
573 motivational state. For example, the call structure of specific 'food calls' in great ape species
574 can vary according to the perceived quality of the food item (e.g. bonobos, *Pan paniscus*,
575 Clay & Zuberbühler, 2009; chimpanzees, Kalan, Mundry, & Boesch, 2015; Slocombe &
576 Zuberbühler, 2006). In this study, we found that hens produced 'Food calls' more often, and
577 at lower peak frequencies, when anticipating the dustbathing substrate than the food rewards.
578 'Fast clucks' were also more prevalent in anticipation of the dustbathing substrate, and
579 produced at lower frequencies in anticipation of both the dustbath and mealworm rewards
580 than the neutral events. This suggests that, in hens, motivational information is contained
581 within the acoustic structure of different call types, as well as within the call type itself. If we
582 group together 'Food calls' and 'Fast clucks' as call types made in anticipation of rewards,
583 this decrease in frequency may indicate further information relating to motivation, such as the
584 signaller's level of arousal. Behavioural data (McGrath et al., 2016) suggest a hierarchy of
585 arousal state, with increased goal-directed behaviour (pushing and pecking against the door),
586 higher activity levels and more approach behaviours demonstrated in anticipation of a
587 dustbathing substrate than the other rewards, and in anticipation of all rewards than neutral
588 treatments. Interestingly, in animals such as baboons, *Papio hamadrayas ursinus* (Rendall,
589 2003), pigs, *Sus scrofa* (Puppe, Schön, Tuchscherer, & Manteuffel, 2005) and domestic cats,
590 *Felis catus* (Yeon et al., 2011), the acoustic frequency of calls increases in parallel with
591 heightened arousal, and this pattern is in accordance with Morton's motivation structural rules

592 (Morton, 1977). However, when approached by humans, tame silver foxes, *Vulpes vulpes*,
593 produce calls at lower peak frequencies (based on the fundamental frequency) than aggressive
594 (untamed) silver foxes (Gogoleva, Volodin, Volodina, Kharlamova, & Trut, 2010), suggesting
595 that a lower peak frequency reflects a positively valenced high arousal state rather than a low-
596 arousal feeling of contentment. Therefore, if we assume that lower acoustic frequency calls
597 reflect a reward-related high arousal state in hens, then call frequency, as well as the call types
598 identified in this study, could be used as a marker of welfare.

599

600 One potential issue with our study was that the dustbathing substrate could have been viewed
601 by hens as an opportunity to forage. Hens were deprived of food for 1.5 h to ensure that they
602 were motivated to eat once they had access to food. As hens dustbathe every 2 days on
603 average (Vestergaard, 1982), we deprived the hens of dustbathing substrate for a longer (24 h)
604 period. Chickens tend not to dustbathe during the morning hours, and the fact that some hens
605 dustbathed immediately after accessing the reward chamber suggests that they were highly
606 motivated to do so (testing had ended by 1230 hours on each day). Based on the behavioural
607 data, it seems that they were motivated to use the dustbathing substrate for exactly this
608 purpose rather than for foraging, although the two potential opportunities are not mutually
609 exclusive. A dustbathing substrate can be a multifaceted resource for hens, including as a
610 potential nest site in a caged environment (Smith, Appleby, & Hughes, 1993), and scratching,
611 foraging and pecking in litter are not only precursors to dustbathing, but also behaviours that
612 hens are motivated to perform in their own right (Olsson & Keeling, 2005). None of our hens
613 performed nesting behaviour during the trials in our experiment, and based on published
614 behavioural results (McGrath et al., 2016) we suggest that anticipation of a dustbathing
615 substrate induced high arousal in hens, indicating they were motivated to dustbathe.

616

617 Another limitation of this study is that we cannot definitively attribute acoustic variation to
618 any particular valence or emotional state. Indeed, Briefer (2012) emphasized that vocal
619 correlates of positive valence are lacking in the literature, often due to a lack of opportunity to
620 record vocalizations in positive situations. Detecting valence is possible if studies use
621 situations of contrasting valence but similar arousal, and studies on dogs (Taylor, Reby, &
622 McComb, 2009) squirrel monkeys, *Saimiri sciurius* (Fichtel, Hammerschmidt, & Jürgens,
623 2001) and goats, *Capra hircus* (Briefer, Tettamanti, & McElligott, 2015) have successfully
624 separated the effects of arousal and valence on vocal parameters. Measuring acoustic
625 parameters such as formants, frequency range and frequency modulation (Briefer, 2012;
626 Briefer et al., 2015) in conjunction with behavioural and physiological measurements may
627 provide the most convincing evidence for valence. For example, different ultrasonic call types
628 made by rats have been linked to activation of positive or negative internal states in both
629 signallers and receivers (Brudzynski, 2007; Burman, Ilyat, Jones, & Mendl, 2007). As our
630 study used items that are known to be rewarding to chickens, we can hypothesize that shifts to
631 a lower peak frequency within 'Food calls' and 'Fast clucks' in anticipation of rewards
632 reflects an increase in positively valenced arousal. The function of this affective response, as
633 suggested by Mendl et al. (2010), could be to guide animals towards acquisition of fitness-
634 enhancing rewards. Hence, future investigations could focus on how conspecifics perceive
635 these vocalizations. Furthermore, there is scope for studies to simultaneously investigate
636 vocal, behavioural and physiological indicators of arousal and valence in chickens.

637

638 <H2>Conclusions

639 This is the first study to find that hens' vocalizations may contain motivational information in
640 call types and call structure. Moreover, the 'Food call' was not given exclusively in response
641 to cues signalling food rewards, suggesting this call was not functionally referential but more
642 likely to be a 'Reward call'. Further work, using other types of reward, will help determine if

643 this is the case. In addition, confirmation of whether males and females produce acoustically
644 different 'Reward calls' would help to assess the function of these calls. Linking
645 physiological and behavioural responses of hens with changes in vocal parameters when
646 anticipating rewards will further identify whether levels of signaller arousal and valence are
647 encoded within the parameters of their calls. Overall, the results of this study suggest that the
648 vocal behaviour of hens can provide an indicator of whether they are in a rewarding
649 environment. These findings can be developed into a tool that could provide a relatively easy
650 method with which to assess the welfare of hens. In a longer study, this tool could monitor
651 welfare outcomes as a result of environmental improvements that may also enhance health
652 and productivity.

653

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656

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Table 1: Hen cue groups and combinations of CS and US

Groups	Mealworm	Dustbathing substrate	Normal food	Sound-neutral
Cue group 1 (<i>N</i> =3)	Ring	Beep	Buzz	Horn
Cue group 2 (<i>N</i> =3)	Horn	Ring	Beep	Buzz
Cue group 3 (<i>N</i> =3)	Buzz	Horn	Ring	Beep
Cue group 4 (<i>N</i> =3)	Beep	Buzz	Horn	Ring

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Table 2: Description of the measurements used in the quantitative classification of call types

Measurement	Description
Call duration	Time (s) from beginning of first syllable to the end of the last syllable in the sequence
Number of syllables	Number of syllables in the call
Average syllable length	Mean length (s) of syllables in a call
Maximum syllable length	Length of the syllable with the longest duration within the call (s)
Minimum Frequency	The lowest frequency bound of the syllable (Hz)
Maximum Frequency	The highest frequency bound of the syllable in (Hz)
Q1 Frequency	The frequency that divides the fundamental frequency into two frequency intervals containing 25% and 75% of the energy (Hz)
Q3 Frequency	The frequency that divides the fundamental frequency into two frequency intervals containing 75% and 25% of the energy (Hz)
Centre Frequency	The frequency that divides the fundamental frequency into two frequency intervals of equal energy (Hz)
Peak Frequency	The frequency at which the maximum power (dB) occurs within the fundamental frequency component (Hz)
Delta Frequency	Difference between the lowest and upper limits of frequency (Hz)
Bandwidth 90%	Difference between the 5% and 95% frequencies (Hz)
Interquartile (IQR) Bandwidth	Difference between the 1st and 3rd Quartile Frequencies (Hz)

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All acoustic measurements are measured on the fundamental frequency component.

933 Table A1
 934 Number of call types made by birds
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Bird	Food call	Fast cluck	Whine	Gakel call	Singing	Mixed	Double cluck	Single cluck	Total no. of calls made
1	1	13	0	7	0	19	6	5	51
2	5	4	4	10	2	3	1	6	35
3	1	5	11	1	2	9	8	11	48
4	3	16	0	0	1	11	10	17	58
5	24	3	1	0	0	9	10	11	58
6	4	6	0	10	0	12	5	5	42
7	2	0	16	5	2	4	0	0	29
8	4	5	9	0	0	16	1	1	36
9	0	4	11	1	0	7	12	16	51
10	6	7	3	2	7	9	1	2	37
11	8	6	2	8	1	11	5	6	47
12	2	6	19	9	1	14	1	2	54
Totals	60	75	76	53	16	124	60	82	

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Table A2

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Mean values of acoustic parameters by call type

Description	Peak Frequency (Hz)		Min Frequency (Hz)		Max frequency (Hz)		Centre Frequency (Hz)		Q1 Frequency (Hz)		Q3 Frequency (Hz)		Delta Frequency (Hz)		Bandwidth 90% (Hz)		IQR Bandwidth (Hz)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Single cluck	340.6	112.3	261.4	110.2	433.8	121.2	342.7	111.8	324.3	111.2	361.6	112.2	171.8	39.3	97.9	30.4	37.3	13.9
Double cluck	389.2	122.4	277.2	108.0	516.6	151.3	390.8	122.2	372.0	120.4	410.9	124.7	165.6	33.2	96.4	26.7	38.8	14.3
Fast cluck	411.6	132.8	244.8	125.8	609.9	194.1	412.2	133.7	393.0	130.4	432.2	136.7	162.9	41.3	95.8	29.0	39.2	13.4
Food call	457.6	84.2	318.5	98.8	620.3	118.5	459.1	84.3	438.5	83.8	479.8	84.2	173.7	30.1	101.7	26.8	41.4	14.4
Mixed	474.6	137.6	287.4	138.0	694.6	184.5	474.9	136.9	452.9	135.2	497.5	138.8	192.4	56.4	108.6	38.4	44.5	21.4
Whine	621.4	137.7	401.4	145.4	869.4	148.1	617.9	128.3	591.4	126.9	641.8	130.7	274.0	78.1	130.0	54.4	50.4	25.9
Singing	644.6	107.6	337.2	108.5	937.3	127.3	638.1	100.0	589.2	95.9	679.3	106.4	366.8	123.8	218.7	94.1	90.2	41.5
Gakel call	502.6	96.6	313.7	74.7	733.2	179.0	498.7	93.4	473.4	86.4	521.1	100.4	245.6	99.5	120.5	58.6	47.9	34.0

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Comment [NM1]: I have addressed both these comments now.

Comment [AT2]: Author: Delete the full stop after 'Hz'.

Comment [AT3]: Author: you refer to a Harsh call in this table. Do you mean Gakel call? If so, change it.

Table A3
Vocal parameters: lsmeans, 95% confidence limits and significance levels

Treatment		Dusty substrate			Mealworm			Normal food			Sound-neutral			Muted-neutral			χ^2	$\chi^2 df$	P
Vocalization type	Parameter	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL			
Food call	PF	411.85 ^a	383.86	441.88	487.16 ^b	451.36	525.80	467.55 ^{ab}	431.98	506.04							8.78	2	0.01
	BW 90%	95.24	90.44	100.29	96.44	90.14	103.18	94.70	88.15	101.73							0.04	2	0.98
	SL	0.10	0.09	0.11	0.10	0.09	0.11	0.10	0.09	0.11							0.22	2	0.89
	CD	0.86	0.77	0.96	1.05	0.92	1.21	1.02	0.88	1.18							2.04	2	0.36
Fast cluck	PF	402.66 ^a	372.47	435.29	403.12 ^a	369.26	440.08	409.19 ^{ab}	374.72	446.84	456.59 ^{ab}	412.44	505.46	548.59 ^b	490.62	613.40	11.30	4	0.02
	BW 90%	90.04 ^a	85.06	95.32	98.86 ^{ab}	92.08	106.14	89.96 ^a	83.74	96.63	86.71 ^a	79.33	94.78	126.81 ^b	114.72	140.18	13.25	4	0.01
	SL	0.09	0.08	0.10	0.09	0.09	0.10	0.10	0.09	0.11	0.08	0.07	0.09	0.09	0.08	0.10	5.34	4	0.25
	CD	0.76	0.69	0.84	0.66	0.59	0.75	0.73	0.65	0.83	0.80	0.69	0.92	0.87	0.74	1.02	3.51	4	0.48
Whine	PF	574.61	530.93	621.87	628.35	578.13	682.94	630.57	585.21	679.44	644.15	600.14	691.38	642.80	597.85	691.12	4.01	4	0.40
	BW 90%	128.11	112.75	145.55	112.89	97.99	130.04	118.50	105.47	133.14	119.12	107.88	131.53	129.27	116.26	143.74	1.25	4	0.87
	SL	0.42	0.34	0.51	0.36	0.29	0.46	0.47	0.39	0.57	0.47	0.39	0.56	0.56	0.46	0.68	5.70	4	0.22
	CD	1.38	1.19	1.60	1.52	1.31	1.77	1.47	1.29	1.68	1.58	1.38	1.80	1.49	1.30	1.70	1.64	4	0.80
Gakel call	PF				481.99	450.38	515.82	502.85	470.60	537.31	474.48	444.98	505.95	484.51	455.36	515.52	1.41	3	0.70
	BW 90%				96.74	82.85	112.95	113.47	97.66	131.84	106.75	92.64	123.00	113.82	99.45	130.27	1.62	3	0.65
	SL				0.61	0.49	0.76	0.65	0.53	0.81	0.70	0.57	0.86	0.76	0.63	0.93	1.61	3	0.66
	CD				2.42	2.03	2.88	2.52	2.13	2.99	3.12	2.67	3.64	2.62	2.26	3.03	2.36	3	0.50
Double cluck	PF	351.20	320.54	384.80	369.98	333.79	410.10	456.35	406.47	512.35	460.03	402.85	525.33	406.20	350.10	471.29	10.95	4	0.03
	BW 90%	93.46	87.60	99.72	88.15	81.43	95.42	95.82	86.69	105.90	96.32	85.09	109.03	95.29	82.55	109.98	0.97	4	0.91
	SL	0.09	0.09	0.10	0.08	0.07	0.08	0.09	0.08	0.11	0.09	0.08	0.11	0.11	0.09	0.13	7.04	4	0.13
	CD	0.33	0.31	0.36	0.27	0.25	0.30	0.32	0.28	0.36	0.29	0.25	0.33	0.32	0.27	0.38	3.62	4	0.46
Single cluck	PF	331.84	303.89	362.36	310.08	277.22	346.83	350.65	315.74	389.43	289.94	262.10	320.74	343.98	311.70	379.60	4.86	4	0.30
	BW 90%	89.12	84.13	94.40	83.44	76.11	91.47	99.37	91.49	107.92	89.04	82.34	96.28	111.01	102.84	119.84	8.78	4	0.07
	SL	0.09	0.08	0.10	0.09	0.08	0.10	0.09	0.08	0.10	0.07	0.06	0.08	0.08	0.07	0.09	7.92	4	0.09
	CD	0.09	0.08	0.10	0.09	0.08	0.10	0.09	0.08	0.10	0.07	0.06	0.08	0.08	0.07	0.09	7.91	4	0.09

945 PF = Peak Frequency, BW 90% = Bandwidth 90%, SL = syllable length, CD = Call duration. Bold indicates significant P values.

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Table A4

Comparison of the different linear mixed-effects models (LMMs) for behavioural responses

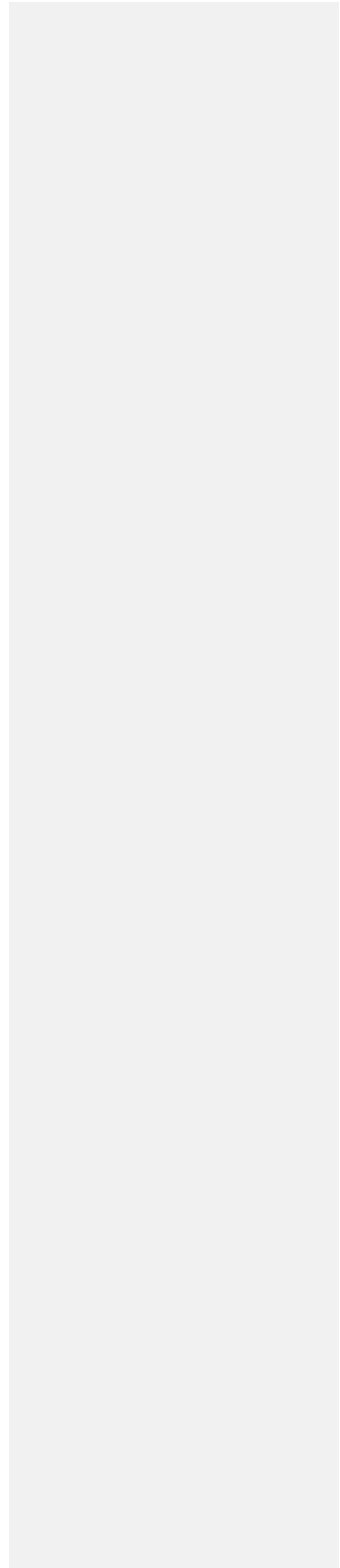
Comment [AT4]: Author: we do not divide tables into parts a and b. You must make these two tables into one single table. There must be a single title above the table and a single footnote at the bottom of the table. The footnote should be a single paragraph. Do not repeat the column headings within the table.

Comment [AT5]: Author: you need to add in the footnote why some variables are in bold type.

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Parameter	Call Type	Fixed variables in model	Comparison	df	AIC	χ^2	$\chi^2 df$	P-value
Peak Frequency	Fast cluck	Treatment		7	-110			
		Treatment + CG	1 vs 2	10	-109	5.62	3	NS
		Treatment + CG+ Day	2 vs 3	14	-106	4.30	4	NS
Bandwidth 90%	Fast cluck	Treatment		7	-113			
		Treatment + CG	1 vs 2	10	-112	4.60	3	NS
		Treatment + CG+ Day	2 vs 3	14	-106	1.56	4	NS
Average syllable length	Fast cluck	Treatment		7	-110			
		Treatment + CG	1 vs 2	10	-110	6.46	3	NS
		Treatment + CG+ Day	2 vs 3	14	-116	14.28	4	0.01
Duration	Fast cluck	Treatment		7	-47			
		Treatment + CG	1 vs 2	10	-45	4.12	3	NS
		Treatment + CG+ Day	2 vs 3	14	-42	4.97	4	NS
Peak Frequency	Food call	Treatment		7	-99			
		Treatment + CG	1 vs 2	10	-98	5.71	3	NS
		Treatment + CG+ Day	2 vs 3	14	-93	2.73	4	NS
Bandwidth 90%	Food call	Treatment		7	-74			
		Treatment + CG	1 vs 2	10	-69	1.84	3	NS
		Treatment + CG+ Day	2 vs 3	14	-67	5.87	4	NS
Average syllable length	Food call	Treatment		7	-57			
		Treatment + CG	1 vs 2	10	-53	1.70	3	NS
		Treatment + CG+ Day	2 vs 3	14	-45	0.56	4	NS
Duration	Food call	Treatment		7	-20			
		Treatment + CG	1 vs 2	10	-19	4.47	3	NS
		Treatment + CG+ Day	2 vs 3	14	-11	0.17	4	NS
Peak Frequency	Gakel call	Treatment		7	-134			
		Treatment + CG	1 vs 2	10	-129	0.63	3	NS
		Treatment + CG+ Day	2 vs 3	14	-123	2.33	4	NS
Bandwidth 90%	Gakel call	Treatment		7	-30			
		Treatment + CG	1 vs 2	10	-27	3.79	3	NS
		Treatment + CG+ Day	2 vs 3	14	-22	2.78	4	NS
Average syllable length	Gakel call	Treatment		7	4			
		Treatment + CG	1 vs 2	10	2	7.98	3	0.05
		Treatment + CG+ Day	2 vs 3	14	5	4.38	4	NS
Duration	Gakel call	Treatment		7	-5			
		Treatment + CG	1 vs 2	10	0	1.87	3	NS
		Treatment + CG+ Day	2 vs 3	14	3	4.44	4	NS
Peak Frequency	Whine	Treatment		7	-162			
		Treatment + CG	1 vs 2	10	-157	0.65	3	NS
		Treatment + CG+ Day	2 vs 3	14	-157	7.92	4	NS
Bandwidth 90%	Whine	Treatment		7	-47			
		Treatment + CG	1 vs 2	10	-42	1.38	3	NS
		Treatment + CG+ Day	2 vs 3	14	-38	3.69	4	NS
Average syllable length	Whine	Treatment		7	-1			
		Treatment + CG	1 vs 2	10	1	4.24	3	NS
		Treatment + CG+ Day	2 vs 3	14	1	8.05	4	NS
Duration	Whine	Treatment		7	-81			
		Treatment + CG	1 vs 2	10	-80		3	NS
		Treatment + CG+ Day	2 vs 3	14	-86	13.41	4	0.01
Peak Frequency	Single cluck	Treatment		7	-102			
		Treatment + CG	1 vs 2	10	-96	0.91	3	NS
		Treatment + CG+ Day	2 vs 3	14	-95	6.69	4	NS
Bandwidth 90%	Single cluck	Treatment		7	-104			
		Treatment + CG	1 vs 2	10	-102	4.54	3	NS
		Treatment + CG+ Day	2 vs 3	14	-98	3.27	4	NS
Average syllable length	Single cluck	Treatment		7	-62			
		Treatment + CG	1 vs 2	10	-58	2.19	3	NS
		Treatment + CG+ Day	2 vs 3	14	-60	9.90	4	0.04
Duration	Single cluck	Treatment		7	-62			
		Treatment + CG	1 vs 2	10	-58	2.19	3	NS
		Treatment + CG+ Day	2 vs 3	14	-60	9.90	4	0.04
Peak Frequency	Double cluck	Treatment		7	-82			
		Treatment + CG	1 vs 2	10	-83	6.34	3	NS
		Treatment + CG+ Day	2 vs 3	14	-81	6.14	4	NS
Bandwidth 90%	Double cluck	Treatment		7	-80			
		Treatment + CG	1 vs 2	10	-74	0.31	3	NS
		Treatment + CG+ Day	2 vs 3	14	-72	5.69	4	NS
Average syllable length	Double cluck	Treatment		7	-55			
		Treatment + CG	1 vs 2	10	-58	9.15	3	0.03
		Treatment + CG+ Day	2 vs 3	14	-59	8.82	4	NS

951 The table shows Akaike information criterion (AIC)
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 959 Table A5
 960 Food call: final model output from LMM
 961

Model	Estimate	SE	t	Pr(> t)
Peak Frequency~Treatment+1 CG:Bird				
<i>df</i> (5),AIC -78.6				
(Intercept) Dustbathing substrate	2.615	0.0288		
Mealworm	0.073	0.0231	3.16	0.00
Normal food	0.055	0.0256	2.16	0.04
Bandwidth 90%~Treatment+1 CG:Bird				
<i>df</i> (5),AIC -66.5				
(Intercept) Dustbathing substrate	1.979	0.0203		
Mealworm	0.005	0.0340	0.16	0.87
Normal food	-0.002	0.0351	-0.07	0.94
Syllable Length~Treatment+1 CG:Bird				
<i>df</i> (5),AIC -53.9				
(Intercept) Dustbathing substrate	-1.008	0.0299		
Mealworm	0.010	0.0367	0.28	0.79
Normal food	-0.011	0.0397	-0.27	0.79
Call Duration~Treatment+1 CG:Bird				
<i>df</i> (5),AIC -17.5				
(Intercept) Dustbathing substrate	-0.066	0.0433		
Mealworm	0.089	0.0633	1.41	0.17
Normal food	0.075	0.0670	1.12	0.27

962
 963 Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the
 964 dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the
 965 estimates in each model being the difference from the intercept. The estimates refer to the values given
 966 using a log-transformed dependent variable.
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 983 Table A6
 984 Fast cluck: final model output from LMM
 985

Model	Estimate	SE	<i>t</i>	Pr(> <i>t</i>)
Peak Frequency~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -109.7				
(Intercept) Muted neutral	2.739	0.0464		
Dustbathing substrate	-0.134	0.0425	-3.16	0.00
Mealworm	-0.134	0.0435	-3.08	0.00
Sound neutral	-0.080	0.0490	-1.63	0.11
Normal food	-0.127	0.0460	-2.77	0.01
Bandwidth 90%~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -113.4				
(Intercept) Muted neutral	2.103	0.0413		
Dustbathing substrate	-0.149	0.0432	-3.44	0.00
Mealworm	-0.108	0.0452	-2.39	0.02
SN	-0.165	0.0505	-3.27	0.00
Normal food	-0.149	0.0471	-3.17	0.00
Syllable				
Length~Treatment+CG+Day+1 CG:Bird				
<i>df</i> (14),AIC -116.2				
(Intercept) Muted neutral	-1.050	0.0506		
Dustbathing substrate	-0.008	0.0395	-0.20	0.84
Mealworm	0.003	0.0410	0.06	0.95
Sound neutral	-0.047	0.0461	-1.02	0.31
Normal food	0.045	0.0433	1.03	0.31
CG2	0.020	0.0460	0.44	0.67
CG3	0.036	0.0513	0.69	0.50
CG4	0.123	0.0437	2.82	0.02
Day2	-0.032	0.0323	-1.00	0.32
Day3	-0.082	0.0314	-2.61	0.01
Day4	-0.092	0.0374	-2.45	0.02
Day5	0.033	0.0351	0.93	0.36
Call Duration~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -47.1				
(Intercept) Muted neutral	-0.059	0.0660		
Dustbathing substrate	-0.062	0.0664	-0.93	0.35
Mealworm	-0.118	0.0688	-1.72	0.09
Sound neutral	-0.040	0.0771	-0.51	0.61
Normal food	-0.075	0.0722	-1.04	0.30

986
 987 Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the
 988 dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the

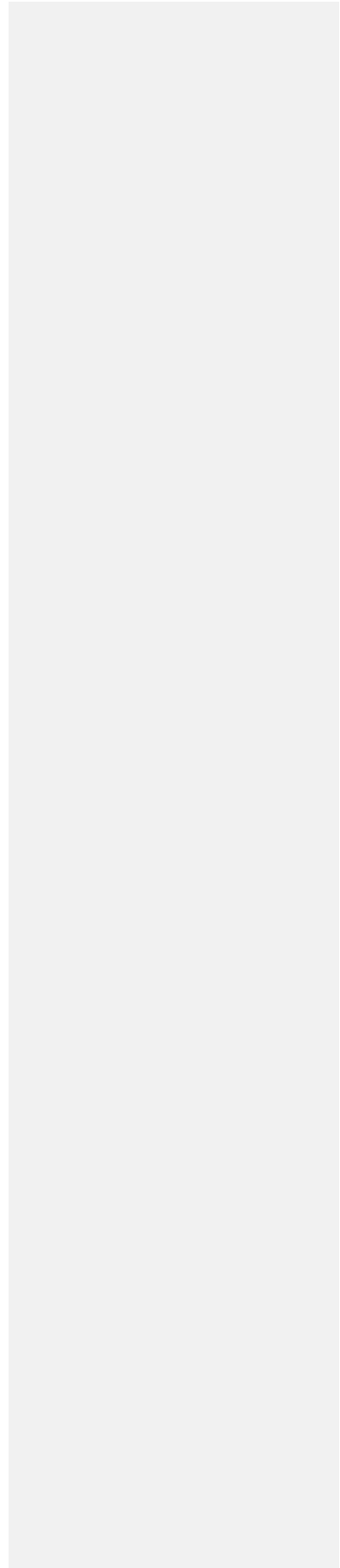
989 estimates in each model being the difference from the intercept. The estimates refer to the values given
990 using a log-transformed dependent variable.
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 993 Table A7
 994 Whine: final model output from LMM
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Model	Estimate	SE	t	Pr(> t)
Peak Frequency~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -161.93				
(Intercept) Muted neutral	2.808	0.0295		
Dustbathing substrate	-0.049	0.0265	-1.84	0.07
Mealworm	-0.010	0.0284	-0.35	0.73
Sound neutral	0.001	0.0221	0.04	0.97
Normal food	-0.008	0.0245	-0.34	0.73
Bandwidth 90%~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -47.0				
(Intercept) Muted neutral	2.112	0.0424		
Dustbathing substrate	-0.004	0.0591	-0.07	0.95
Mealworm	-0.059	0.0646	-0.91	0.37
Sound neutral	-0.036	0.0500	-0.71	0.48
Normal food	-0.038	0.0555	-0.68	0.50
Syllable Length~Treatment+CG+1 CG:Bird				
<i>df</i> (7),AIC -1.2				
(Intercept) Muted neutral	-0.252	0.0765		
Dustbathing substrate	-0.129	0.0773	-1.67	0.10
Mealworm	-0.185	0.0831	-2.23	0.03
Sound neutral	-0.077	0.0646	-1.19	0.24
Normal food	-0.074	0.0716	-1.03	0.31
Call Duration~Treatment+CG+Day+1 CG:Bird				
<i>df</i> (14),AIC -85.7				
(Intercept) Muted neutral	0.270	0.0781		
Dustbathing substrate	-0.032	0.0413	-0.78	0.44
Mealworm	0.010	0.0449	0.22	0.83
Sound neutral	0.026	0.0363	0.72	0.47
Normal food	-0.004	0.0392	-0.10	0.92
CG2	-0.156	0.1542	-1.01	0.32
CG3	-0.203	0.0838	-2.43	0.04
CG4	-0.209	0.0881	-2.37	0.04
Day2	0.086	0.0437	1.96	0.05
Day3	0.065	0.0492	1.33	0.19
Day4	-0.021	0.0372	-0.57	0.57
Day5	0.089	0.0412	2.15	0.03

996
 997 Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the
 998 dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the
 999 estimates in each model being the difference from the intercept. The estimates refer to the values given
 1000 using a log-transformed dependent variable.

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1003
 1004 Table A8
 1005 Gakel call: final model output from LMM
 1006

Model	Estimate	SE	t	Pr(> t)
Peak Frequency~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -133.9				
(Intercept) Muted neutral	2.685	0.0253		
Mealworm	-0.002	0.0215	-0.11	0.92
Sound neutral	-0.009	0.0190	-0.48	0.63
Normal food	0.016	0.0213	0.76	0.45
Bandwidth 90% ~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -29.6				
(Intercept) Muted neutral	2.056	0.0547		
Mealworm	-0.071	0.0604	-1.17	0.25
Sound neutral	-0.028	0.0534	-0.52	0.60
Normal food	-0.001	0.0594	-0.02	0.98
Syllable Length~Treatment+1 CG:Bird				
<i>df</i> (10),AIC 1.8				
(Intercept) Muted neutral	-0.279	0.0921		
Mealworm	-0.096	0.0793	-1.21	0.23
Sound neutral	-0.037	0.0701	-0.52	0.60
Normal food	-0.066	0.0774	-0.85	0.40
CG2	0.044	0.1511	0.29	0.78
CG3	0.513	0.1460	3.51	0.00
CG4	0.090	0.1166	0.77	0.46
Call Duration~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -4.6				
(Intercept) Muted neutral	0.418	0.0590		
Mealworm	-0.034	0.0799	-0.43	0.67
Sound neutral	0.076	0.0707	1.08	0.29
Normal food	-0.016	0.0776	-0.21	0.84

1007
 1008 Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the
 1009 dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the
 1010 estimates in each model being the difference from the intercept. The estimates refer to the values given
 1011 using a log-transformed dependent variable.
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 1022 Table A9
 1023 Single cluck: final model output from LMM
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Model	Estimate	SE	t	Pr(> t)
Peak Frequency~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -101.5				
(Intercept) Muted neutral	2.537	0.0406		
Dustbathing substrate	-0.016	0.0358	-0.44	0.66
Mealworm	-0.045	0.0475	-0.95	0.35
Sound neutral	-0.074	0.0417	-1.78	0.08
Normal food	0.008	0.0450	0.185	0.85
Bandwidth 90% ~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -103.8				
(Intercept) Muted neutral	2.045	0.0309		
Dustbathing substrate	-0.095	0.0374	-2.55	0.01
Mealworm	-0.124	0.0482	-2.57	0.01
Sound neutral	-0.096	0.0431	-2.22	0.03
Normal food	-0.048	0.0448	-1.074	0.29
Syllable				
Length~Treatment+CG+Day+1 CG:Bird				
<i>df</i> (14),AIC -59.7				
(Intercept) Muted neutral	-1.167	0.0623		
Dustbathing substrate	0.053	0.0468	1.13	0.26
Mealworm	0.046	0.0597	0.77	0.44
Sound neutral	-0.073	0.0531	-1.38	0.17
Normal food	0.045	0.0556	0.806	0.42
CG2	0.011	0.0541	0.194	0.85
CG3	-0.009	0.0683	-0.14	0.89
CG4	0.043	0.0666	0.65	0.53
Day2	-0.003	0.0530	-0.06	0.95
Day3	0.175	0.0690	2.531	0.01
Day4	0.025	0.0452	0.543	0.59
Day5	0.104	0.0506	2.06	0.04
Call Duration~Treatment+1 CG:Bird				
<i>df</i> (10),AIC				
(Intercept) Muted neutral	-1.167	0.0623		
Dustbathing substrate	0.053	0.0468	1.12	0.26
Mealworm	0.046	0.0597	0.77	0.44
Sound neutral	-0.073	0.0531	-1.38	0.17
Normal food	0.045	0.0556	0.805	0.42
CG2	0.011	0.0541	0.195	0.85
CG3	-0.009	0.0683	-0.14	0.89
CG4	0.043	0.0666	0.65	0.52
Day2	-0.003	0.0531	-0.06	0.95

Day3	0.175	0.0691	2.53	0.01
Day4	0.025	0.0453	0.543	0.59
Day5	0.104	0.0506	2.05	0.04

1025
1026 Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the
1027 dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the
1028 estimates in each model being the difference from the intercept. The estimates refer to the values given
1029 using a log-transformed dependent variable.
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1036 Table A10
 1037 Double cluck: final model output from LMM
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Model	Estimate	SE	t	Pr(> t)
Peak Frequency~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -82.4				
(Intercept) Muted neutral	2.609	0.0608		
Dustbathing substrate	-0.063	0.0562	-1.12	0.27
Mealworm	-0.041	0.0544	-0.75	0.46
Sound neutral	0.054	0.0650	0.83	0.41
Normal food	0.051	0.0636	0.795	0.43
Bandwidth 90% ~Treatment+1 CG:Bird				
<i>df</i> (7),AIC -80.1				
(Intercept) Muted neutral	1.979	0.0572		
Dustbathing substrate	-0.008	0.0603	-0.14	0.89
Mealworm	-0.034	0.0609	-0.56	0.58
Sound neutral	0.005	0.0727	0.06	0.95
Normal food	0.002	0.0674	0.036	0.97
Syllable				
Length~Treatment+CG+Day+1 CG:Bird				
<i>df</i> (14),AIC -58.2				
(Intercept) Muted neutral	-0.990	0.0666		
Dustbathing substrate	-0.075	0.0695	-1.08	0.28
Mealworm	-0.162	0.0721	-2.25	0.03
Sound neutral	-0.088	0.0865	-1.02	0.31
Normal food	-0.070	0.0767	-0.916	0.36
CG2	-0.039	0.0425	-0.927	0.36
CG3	0.136	0.0491	2.77	0.01
CG4	0.041	0.0592	0.69	0.49
Call Duration~Treatment+1 CG:Bird				
<i>df</i> (10),AIC -61.0				
(Intercept) Muted neutral	-0.493	0.0676		
Dustbathing substrate	0.016	0.0707	0.22	0.83
Mealworm	-0.069	0.0707	-0.98	0.33
Sound neutral	-0.051	0.0846	-0.60	0.55
Normal food	-0.007	0.0792	-0.085	0.93

1039
 1040 Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the
 1041 dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the
 1042 estimates in each model being the difference from the intercept. The estimates refer to the values given
 1043 using a log-transformed dependent variable.
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Table A11
Random Forest confusion matrix and classification error for each call type

Call type	Double cluck	Fast cluck	Food call	Gakel call	Mixed	Singing	Single cluck	Whine	Classification error
Double cluck	59	0	0	0	0	0	1	0	0.017
Fast cluck	0	49	13	0	13	0	0	0	0.347
Food call	0	17	33	0	10	0	0	0	0.450
Gakel call	0	0	0	38	6	1	0	8	0.283
Mixed	0	19	11	3	74	3	0	14	0.403
Singing	0	0	0	0	7	6	0	3	0.625
Single cluck	0	0	0	0	0	0	82	0	0.000
Whine	0	0	0	6	10	0	0	60	0.211

1048 The OOB error rate was 26.56%. The first column lists call types from our subjective classification by
1049 name, and the following columns show the number of each call type the initial call types were classified
1050 into by Random Forest. The final column shows the classification error rate for each call type.

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1056 Table A12

1057 Gini index showing importance of predictor variables in the Random Forest analysis.

Variable	Mean decrease in Gini
Call Duration	87.52
Max Syllable Length	61.59
Number of Syllables	72.42
Average syllable length	54.83
Peak Frequency	19.69
Minimum Frequency	22.31
Maximum Frequency	27.06
Centre Frequency	20.22
Q1 Frequency	19.57
Q3 Frequency	20.21
Frequency Range	25.26
Bandwidth	17.56
IQR Bandwidth	16.72

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1063 **List of Figures.**

1064 Fig.1. The experimental pen. L shows where the lamp was positioned.

1065 Fig. 2. Number of calls made and number of calls analysed by treatment.

1066 Fig.3. Classification and regression tree of vocalizations made by chickens in anticipation of rewards. The
1067 tree shows the variables used at each split, with the criteria (<,>,<=). Terminal nodes indicate the prediction
1068 of call type for that partition and the number of correctly classified call types out of the total in that
1069 subgroup. ('Food C' = Food call, 'Fast C' = Fast cluck, 'Double C' = Double cluck, 'Single C' = Single
1070 cluck, 'Duration' = Call Duration, 'Timesyll' = Average syllable length, 'Numsyll' = Number of syllables,
1071 'Peak' = Peak Frequency, 'Max' = Maximum Frequency)

1072 Fig. 4. Model predictions of call rate per hen for eight different call types in anticipation of five different
1073 treatments. (a) Food calls and clucks. (b) Other calls. SN: sound-neutral; MN: muted-neutral. Means are
1074 shown with lower and upper confidence limits. * $P<0.05$; ** $P<0.01$; *** $P<0.001$: call rates that were
1075 significantly higher than MN.
1076

1077 Fig. 5. Variation in the peak frequency of 'Food calls' by reward. Central dot indicates mean values and
1078 horizontal lines show SEs.
1079

1080 Fig. 6 Spectrograms of (a) 'Food call', (b) 'Fast cluck', (c) 'Single cluck' and (d) 'Double cluck'. A key
1081 within the 'Food call' spectrogram indicates the syllable length and call duration.
1082

1083 Fig. 7. Example spectrograms of (a) 'Whine' and (b) 'Gakel calls'.
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