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Title: Quantifying Hungry Broiler Breeder Dietary Preferences using a Closed Economy T-Maze Task

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**Abstract:** This study aimed to identify hungry broiler breeders ( $n = 12$ ) preferences for quantitative (Control) or qualitative dietary restriction (QDR) in a closed economy environment. The QDR option was either 3 g calcium propionate/kg total feed ( $n = 6$ ) or 300 g oat hulls/kg total feed ( $n = 6$ ). Quantitatively restricted or QDR portions ensured equal growth regardless of choice. Birds were separately taught a Control diet versus no food and a QDR diet versus no food task to allow each diet's satiating properties to be learnt. Birds had to associate the T-maze coloured arms with dietary outcomes to immediately obtain food. Birds learnt this task easily ( $p < 0.001$ ). A choice between the Control diet and the QDR diet was then offered but neither group demonstrated a diet preference. Study modifications demonstrated this was not a failure to discriminate between the diets per se (the Control diet was strongly preferred under ad libitum conditions ( $p < 0.001$ )) or novel colour combination confusion (the colour associated with food was immediately selected when two novel food versus no food colour combinations were offered ( $p < 0.001$ )). Most birds still failed to show a significant preference when the Control diet quantity was increased by 50% to make it 'obviously' bigger and better. Therefore, it was concluded that the failure to show a dietary preference was due to task learning failure and not necessarily lack of dietary preference. Where a preference was observed it was always for the control diet. Possible reasons for this failure to learn are discussed.

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Our Ref: LB/BB diet pref. AABS

Date: 21<sup>st</sup> May 2011

Dear Prof. Jensen,

**Revision of manuscript (APPLAN-S-10-00363) to Applied Animal Behaviour Science**

Please find attached the revised manuscript for “Quantifying hungry broiler breeder dietary preferences using a closed economy T-maze task”, co-authored by LA Buckley, V Sandilands, BJ Tolcamp and RB D’Eath.

The revised paper meets the legal and ethical requirements of the journal, all authors have agreed to the text for publication, and the appropriate acknowledgements have been made. The paper is not being considered for publication elsewhere.

We hope that you and the referees find the revisions acceptable, and I look forward to hearing from you soon.

Best wishes,

Miss Louise Buckley  
*PhD Student / Research Assistant*

Ref: Applan-D-10-292

Title: Quantifying hungry broiler breeder dietary preferences using a closed economy T-maze task

Nb. All changes to the paper have been highlighted in yellow.

Reponses to referees

**Reviewer 1:**

General notes:

Table 1. This was removed to comply with AABS's figure and table limits. Some additional information has been added to help clarify the methodology further. See new lines: 200 - 206.

Presentation of individual data has been added to sections 3.1. (figure two) referred to in lines 312 and 319 and also in section 3.2. (new lines: 334 – 337) and 3.3. (new lines: 355 – 359).

Some specific comments on results section:

Section 3.2.1. and 3.2.2. : additional information on individual preferences is now provided. This is shown in figure two (referred to in new lines 312 and 319). Some additional information has also been provided in section 3.2. (new lines: 334 - 337) and section 3.3. (new lines 355 – 359).

Figure 4 & 5 referred to in original line 332 are incorrect. These should refer to figures 3 and 4. Our apologies.

Line 371: This section has been amended further to referee 2's comments (see new lines: 375 – 388)

Line 406: New lines 355 – 366 and 405 – 406 amended to improve clarity.

Line 535:Amended (new lines: 535 - 536).

Minor points noted:

Line 258: I think that the referee is assuming to original line 242. This has now been amended (new line: 251).

Line 288: “was” – changed to “were” (new line: 298).

Line 295: Amended to “Results” (new line: 305).

Line: 325: Amended to “phase 1 and 2” (new line: 323).

Line 381: Original text deleted.

Line 487: “either removed along with other sentence amendments as per next comment (new line: 489).

Line 487: Line amended (new line: 489).

Line 553: paragraphs joined up at “however” (new line: 552).

Line 556: commas deleted (new line: 555).

Line 579: “;” deleted (new line: 593).

Line 586: “;” deleted (new line: 600).

## **Reviewer 2:**

General comments:

Paragraph 2. This valid point has now been addressed in the discussion. (new line: 577 - 585)

Introduction:

Line 32 – 33: Amended (new line 32).

Line 44 – 47: Several references removed (new lines: 44 - 45).

Line 49: Amended (new line: 47).

Line 55: Amended (new line: 53).

Materials and methods:

Line 88: Clarified by the additional of a couple of explanatory sentences (new lines: 89 - 90).

Line 194: Our apologies. Table 1 was removed to comply with restrictions of the number of tables and graphs we could include. References to this table have now been removed.

Line 208 – 209: This was essential to the study design as we wanted the birds to ‘live with the consequences of their diet choice’ and we expected the birds to take longer to consume the experimental ration than the control ration so it was necessary for the birds to remain within this pen for a period of time following access to the feed. As each bird received each ration in both pens (the study was balanced within bird for side each diet was accessed (i.e. half the time the experimental diet was in the pen located at the end of the right T-maze arm for the bird and half the time it was to be found in the pen located at the end of the left T-maze arm) so any pen familiarity that was induced was controlled for and should not have had any bearing on the choices made by each bird.

Line 225: Amended (new line: 234).

Line 229 – 237: Clarified (new lines: 239 - 240).

Lines 255 – 239: removed information referred to (new lines: 243 - 246)

Data reanalysed without threshold/truncation therefore reference to this has been removed and discussed in the discussion (new lines: 375 – 384)

Line 260: Amended (new line: 267).

Line 260: The rationale for not adopting the methodology: control diet consumed as proportion of control diet available versus fibre diet consumed versus fibre diet available (or CAP as appropriate) is that the quantities of each diet were different. Using the suggested methodology was ruled out by us (although we did come close to adopting this approach) because it led to the counter-intuitive situation in which a bird could consume more fibre diet (g) than the control diet (g) yet, at the same time appear to consume less or the same amount of fibre diet than/as the control diet.

E.g. A bird consumes 5g control and 7g FIBRE diet. – suggests prefers fibre diet

But, adopt the above methodology and this preference disappears due to the disparity in the quantities of each diet offered.

$(5g/11g) * 100 = 45\%$  of control ration consumed

$(7g/15.7g) * 100 = 45\%$  of fibre ration consumed

Throughout the initial two-pan choice test (section 3.2.) the quantities were always identical (i.e. control was always 11g, FIBRE was always 15.7g and CAP was always 11.3g). See new lines 176 - 178). Otherwise, they increase every 7 days by 2g/day (control diet) and proportionately more (CAP and FIBRE diets) to maintain the commercial rate of growth. However, as this increase was minimal when split across 5 portions/day so we do not expect that the birds would have become confused, had their expectations 'not met', etc by this gradual increase over time.

Line 262: Amended (new line: 268)

Line 260 – 266: Unfortunately we did not measure which diet the birds approached first.

Line 267: Information added (new lines: 274).

Line 268: Information added (new lines: 278 - 279).

Line 272: Information added (new line: 280)

Line 279: Information added (new lines: 288 - 289)

Line 285: Information added (new lines: 295 - 296)

Line 297: Deleted information plus removed figure two (growth curve) to allow for individual data in section 3.2. to be added (see new figure two)

Line 313: Data was not collected regarding first diet chosen. Unfortunately, due to labour constraints it was not possible to monitor the birds' initial diet preferences.

Line 309 – 310: The requested information is included in lines 175 – 176 (new line: 178). We have now re-inserted figure 2 which shows individual bird preferences.

Line 337 onwards: We have amended this section to improve clarity. See: new lines: 325 – 327 and 340 - 342).

Line: 356: Amended to read “side biases” (new line: 357).

Line: 364 – 366: This information is included in the methods but we have amended this section of the methods to improve clarity (new lines: 287 - 296).

Discussion:

Line: 371; Line: 378: we have amended the presentation of data by removal of the threshold to present data from all birds. This had minimal effects of the results with no change to the direction and marginal (<1% change) strength of any preference. (See new lines: 264 – 266; 305 – 320; 375 - 384).

Line 378 – 381: See explanation on this document, line 260 above. Analysing the data in this way had no effect on the CVC birds preferences and resulted in a preference for the control diet in the CVF birds. We feel that if this was a ‘true’ preference rather than an artefact of the methodology the preference would remain once analysed by our chosen method.

Experimental modifications:

Line 412: Amended (new lines: 78 – 80).

Line 422 – 423: Amended (new line: 422).

Line 430: Line altered (new lines: 428 – 434)

Line 428 onwards: Amended to improve clarity (new lines: 428 - 434)

Line 449: Amended (new line: 451).

Results (2?): Amended (new line: 465)

Line 473: Amended (new line: 475)

Line 478: Amended (new line: 480)

Line 473: Introduction to this part of the study amended to improve clarity (new line: 428 - 434)

Line 561: Unfortunately latency to consume was not recorded (new line: 560).

Pennington & Thompson used rats in their study (new line: 600).

1 Quantifying Hungry Broiler Breeder Dietary Preferences using a Closed Economy T-Maze  
2 Task

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8 Abstract

9 This study aimed to identify hungry broiler breeders (n = 12) preferences for quantitative  
10 (Control) or qualitative dietary restriction (QDR) in a closed economy environment. The  
11 QDR option was either 3 g calcium propionate/kg total feed (n = 6) or 300 g oat hulls/kg total  
12 feed (n = 6). Quantitatively restricted or QDR portions ensured equal growth regardless of  
13 choice. Birds were separately taught a Control diet versus no food and a QDR diet versus no  
14 food task to allow each diet's satiating properties to be learnt. Birds had to associate the T-  
15 maze coloured arms with dietary outcomes to immediately obtain food. Birds learnt this task  
16 easily (p<0.001). A choice between the Control diet and the QDR diet was then offered but  
17 neither group demonstrated a diet preference. Study modifications demonstrated this was not  
18 a failure to discriminate between the diets per se (the Control diet was strongly preferred  
19 under *ad libitum* conditions (p<0.001)) or novel colour combination confusion (the colour  
20 associated with food was immediately selected when two novel food versus no food colour  
21 combinations were offered (p<0.001)). Most birds still failed to show a significant preference  
22 when the Control diet quantity was increased by 50% to make it 'obviously' bigger and  
23 better. Therefore, it was concluded that the failure to show a dietary preference was due to  
24 task learning failure and not necessarily lack of dietary preference. Where a preference was  
25 observed it was always for the control diet. Possible reasons for this failure to learn are  
26 discussed.

27 Key words: Broiler Breeder; quantitative feed restriction; qualitative food restriction; choice  
28 tests; preference testing; hunger stress

29

## 30 1. Introduction

31 Freedom from hunger is one of the five freedoms necessary for good welfare (FAWC,  
32 1998). Hunger is 'a negative affective state' (D'Eath *et al.*, 2009), associated with suffering  
33 for the animal involved (Dawkins, 1990) However, for broiler breeders (the parent stock of  
34 meat chickens) selectively bred for fast growth (and therefore large appetites), preventing  
35 hunger by *ad libitum* feeding causes obesity and severely compromises physical health and  
36 fertility (Robinson and Wilson, 1996; Hocking *et al.*, 1987). Consequently, optimising  
37 growth through quantitative feed restriction is integral to management in the industry. Birds  
38 are fed 25 – 50% of *ad libitum* intake (Savory *et al.*, 1993). Behavioural and/or physiological  
39 stress indicators are apparent (de Jong *et al.*, 2003; de Jong *et al.*, 2002; Hocking *et al.*, 1996;  
40 Hocking *et al.*, 1993) with general acceptance that these birds experience chronic hunger (de  
41 Jong *et al.*, 2003; Mench, 2002).

42 To address this welfare issue, researchers have attempted to reduce hunger by adjusting the  
43 commercial ration quality either by adding non- or low-nutritive fillers to make the diet more  
44 bulky and / or by adding appetite-suppressing compounds (Sandilands *et al.*, 2005; Hocking  
45 *et al.*, 2004; Nielsen *et al.*, 2003; Savory *et al.*, 1996; Hocking and Bernard, 1993). This is  
46 called qualitative dietary restriction (QDR). It is possible with this method for birds to be fed  
47 *ad libitum*, meet commercial growth rates and be healthy and fertile by adding increased  
48 levels of calcium propionate (CAP) (appetite-suppressing compound) and fixed levels of oat  
49 hulls (fibrous filler) to the commercial ration (Tolkamp *et al.*, 2005).

50 Unfortunately, QDR effects on behavioural and physiological indicators of hunger stress in  
51 feed restricted broilers are mixed (Hocking, 2006; Sandilands *et al.*, 2006; Sandilands *et al.*,  
52 2005; de Jong *et al.*, 2005; Nielsen *et al.*, 2003; Savory and Lariviere, 2000; Savory *et al.*,



53 1996) and studies are inconclusive. A voluntary reduction in overall energy consumed  
54 (compared with *ad libitum* intake of a regular commercial feed) or consumption rate is not  
55 necessarily indicative of, or synonymous with, reduced hunger. Birds may consume less  
56 energy because they are satiated (a positive welfare outcome) or they may eat less or more  
57 slowly because they find the diet aversive (a negative welfare outcome). Further, while  
58 combining CAP and oat hulls has synergistic effects on reducing energy intake (Tolkamp *et*  
59 *al.*, 2005), one compound may be aversive whilst the other satiety-enhancing. Thus,  
60 interpreting differential rates of consumption and other behavioural indicators is difficult  
61 (D'Eath *et al.*, 2009). Consequently, additional methods of quantifying the potential benefits  
62 of feeding QDRs are needed.

63 Choice tests are a novel way to navigate round this interpretive difficulty (D'Eath *et al.*,  
64 2009). Choice tests are widely used in evaluating animal welfare and assume an animal's  
65 preferred option would lead to enhanced welfare. Dawkins (2004) claims only two questions  
66 need answering when evaluating an animal's welfare: Is it healthy? Does it have what it  
67 wants? Healthy broiler breeders can be produced on a typical quantitative restriction diet or  
68 on a QDR (Tolkamp *et al.*, 2005). Therefore, the remaining question is: do feed restricted  
69 broiler breeders prefer this feed restriction to be quantitative or qualitative?

70 This study's primary aim was to investigate feed-restricted broiler breeder (*Gallus gallus*  
71 *domesticus*) preferences for either quantitative feed restriction or a QDR using a closed  
72 economy T-maze colour-diet association and discrimination task. Two different compounds –  
73 CAP and oat hulls (FIBRE) – were tested separately in choice tests (commercial diet versus  
74 experimental diet) in case of conflicting effects on affective state and thus preference. When  
75 initial results suggested no emerging significant preference, the experiment was modified and  
76 further conditions were imposed to determine whether the results reflected genuine  
77 indifference or a failure to learn the task. The specific hypotheses tested are outlined  
78 separately in the relevant experimental modification sections. Thus, it should be noted that the

79 experimental design, results and initial discussion are described in two sections: firstly, the  
80 original study design and, secondly, the subsequent experimental modifications.

## 81 2. Materials and methods

### 82 2.1. Subjects

83 This study used 24 female Ross 308 broiler breeders, obtained as day old chicks. Birds were  
84 randomly allocated to one of two treatment groups at 35 days. These groups were 1) Control  
85 diet versus CAP diet (CVC, n = 12), and 2) Control diet versus FIBRE diet (CVF, n = 12).  
86 Between groups the experimental protocol was identical except for diets fed from day 35 (see  
87 section 2.3.3., start of two-pan choice test). Before beginning the T-maze choice experiment  
88 (day 42), group-size was reduced (n = 6 per treatment group) by euthanizing the three  
89 heaviest and three lightest birds within each treatment group. Group size was reduced for the  
90 second part of the study for practical reasons (equipment and labour availability).

### 91 2.2. Housing & husbandry

92 Birds were reared according to the producer's recommendations for lighting and heating  
93 (stepwise lighting and heating reductions ~ 23 to 8 hours light from day 11 and 31°C to 20-  
94 22°C from day 25 respectively) (Aviagen, 2006). Birds were group-housed according to body  
95 weight in three groups (n=8) until day 14, then eight smaller groups (n=3) until day 35. To aid  
96 growth management, birds were occasionally switched between groups to ensure similar  
97 bodyweight birds were housed together. On days 28 - 34, the birds were fed separately and  
98 then returned to their group. Birds were housed and tested in the same room. However, birds  
99 were housed in different pens from their test pens to ensure pen familiarity did not influence  
100 choice test behaviour. From day 35, birds were housed individually in test pens (9am – 5pm)  
101 and group-housed in home pens overnight. All pens were 1m x 1m, contained wood shavings  
102 and provided ad libitum water access. Home (group) pens were cleaned weekly. Test pens  
103 were cleaned as needed.

### 104 2.3. Nutrition & feeding

105                   2.3.1. Growth curves

106       Bird growth rate (until week 12) was modelled on the producer recommended Ross 308  
107 broiler breeder growth curve for 5% egg production at 25 weeks (Aviagen, 2007) but slightly  
108 exceeded this recommendation post change to mash diet. Target weight gain (weeks 5 – 12)  
109 was 100 g / week. Actual weight gain was an average ((± standard deviation) 119 g / week (±  
110 12.1 g). Producer guidelines state feed levels once increased should never be decreased  
111 (Aviagen, 2006). Further, sudden diet quantity changes could have affected the birds' learning  
112 about diet-satiating properties. Therefore, although bird growth rate was slightly too fast this  
113 trajectory was maintained.

114                   2.3.2. Starter diet and protocol

115       From day 1 – 34, birds were fed a commercial diet (Laser SP starter Crumb, BOCM Pauls  
116 Ltd., Ipswich, Suffolk). Birds were individually fed additional feed if necessary to ensure  
117 actual bodyweight was close to producer target weight and coefficient of variation between  
118 birds was minimised.

119                   2.3.3. Experimental diet and protocol

120       From day 35, birds were fed two diets (see below for feeding/exposure to diets protocol).  
121 The control diet (both treatments) was a custom-made grower mash (Target Feeds Ltd.,  
122 Whitchurch, Shropshire) and was also the basis for both experimental diets. The mash diet  
123 supplied 150 g crude protein and 11.5 MJ ME per kg of food. The CAP diet was the mash diet  
124 plus 30 g Calcium propionate / kg total feed. The FIBRE diet was the mash diet plus 300 g  
125 finely-ground (4mm) oat hulls / kg total feed. Each experimental diet portion was equivalent  
126 to the control diet portion (g) plus the respective addition. The calcium propionate was  
127 supplied as Luprosil ® salt (BASF, Germany).

128       Diet rations were designed to ensure equivalent growth, based on Tolkamp *et al.*, (2005)  
129 who found that the quantity of basal feed (commercial feed minus CAP and OH) consumed

130 ad lib by their QDR birds was similar to birds fed the commercial feed restricted ration. Initial  
131 dietary preferences were also investigated as initial dietary preferences are modifiable by  
132 post-ingestion feedback (Kyriazakis *et al.*, 1999; Forbes, 1998; Provenza, 1995). Quantities of  
133 the compounds added were less than in Tolcamp, *et al* (2005) . This reflected previous  
134 unpublished findings by the authors that indicated that gradual adjustment to QDR may mean  
135 insufficient energy consumption initially if compound inclusion levels are high. Broiler  
136 breeders are sensitive to restriction severity (Savory *et al.*, 1993; Bokkers and Koene, 2004;  
137 Bokkers *et al.*, 2004), thus, we assumed, should prefer an increase in satiety, even if that  
138 satiety is not complete.

## 139 2.4. Experimental apparatus

### 140 2.4.1. Two – pan choice test – initial dietary preference experiment

141 Test pens (1m x 1m) were solid-sided to prevent visual access to other birds. Food was  
142 provided in D-cup feeders (11.25cm (l) x 6.25cm (w) x 8.75cm (d)). These were attached to  
143 the pen front 10 – 12 cm apart. The water bowl was on the floor in the middle of pen.

### 144 2.4.2. T-maze choice test experiment

145 The experimental apparatus comprised two sections: the T-maze and the two terminal testing  
146 pens that the T-maze arms exited into (Figure 1). The T-maze was of wooden construction.  
147 Interchangeable coloured wooden inserts slotted into each T-maze arm (right/left/end). The  
148 maze height was 40cm. Terminal test pens had a guillotine hatchway situated on the front left  
149 of the pen (25cm x 25cm). The D-cup feeder location ensured its contents were only visible  
150 once the bird had entered the terminal pen. The terminal pens were the same as the pens used  
151 to house the birds outside of the test situation and during the initial dietary preference  
152 experiment. However, to prevent familiarity biasing preference, the individual birds were not  
153 tested in the pen(s) they had previously experienced.

154 FIGURE 1 SHOULD GO ABOUT HERE

155           2.5.    Training and testing

156                   2.5.1.   Handling and socialisation

157    To reduce the potential effects of stress, birds were socialised and habituated to potential  
158    environmental stressors by being handled several times a day (10 – 120s) and by gradually  
159    increasing isolation from other chicks. The latter was initially synonymous with handling (as  
160    above) then involved separation of individual chicks by solid barriers and allowed to find  
161    their way around the barrier to return to their group (Day 8 onwards, 10 – 60 sec, 1 – 5  
162    times/day, 3 times/week) and, finally, by daily solitary feeding (day 28 – 35). Solid barrier  
163    use encouraged exploratory behaviour to reduce the risk of fear or anxiety that might affect  
164    performance during the later T-maze training/testing.

165    From day 21, birds were group-introduced to the T-maze and released into the arms to  
166    explore (for 15 minutes/ twice daily; three times/week). From day 28, birds individually  
167    explored the T-maze and adjacent pens (for 15 minutes /once daily three times /week).  
168    Finally, a radio played daily habituated birds to human voices/noise and to mask unwanted  
169    facility sounds.

170                   2.5.2.   Two-pan choice test – initial dietary preference experiment

171    During days 35 – 41, the primary aim was diet habituation before training/testing as dietary  
172    neophobia reduces intake in fowl (Murphy, 1977). However, it also allowed investigation of  
173    initial dietary preferences prior to potential preference modification by post-ingestive effects.

174    Birds were given equal exposure to both the control and experimental diets. Both diets  
175    were offered simultaneously (each portion equalled 1/4 of total daily feed provided) with two  
176    feeding opportunities/day (09:00 h and 13:00 h) for 7 days. The rations offered at each  
177    feeding opportunity over this period were: control diet: 11g; CAP diet 11.3g and FIBRE diet:  
178    15.7g. Individual feed intake was measured twice daily for the first 5 days. The food  
179    removed, weighed, and returned after 10, 20, 30, 40, 50, 60, 120, 180 and 210 min. Food left

180 after 240 min was removed, weighed and discarded. Diet was balanced (within and between  
181 birds) for pen side and randomly switched sides between feeding opportunities.

### 182 2.5.3. T – maze choice test experiment

#### 183 2.5.3.1. General testing protocol

184 Each bird was given five T-maze trials / day (90 min apart). Within treatment group, birds  
185 were tested in the same order each trial. Within trial, all birds in a treatment group were tested  
186 before the other treatment group birds were tested. This was done for practical reasons as  
187 alternating between birds from different treatment groups would have increased the time  
188 taken to test all 12 birds. The group tested first alternated daily. Each bird obtained 1/5 of her  
189 daily feed ration at each trial. No further food was available.

#### 190 2.5.3.2. Dietary contingencies associated with colours

191 The aim of this training was for birds to associate coloured T-maze arms with different  
192 dietary outcomes. Different colours were used for the treatment groups as the experiment was  
193 originally planned as a crossover design. Necessary experimental modifications prevented this  
194 crossover and it is not referred to further. The colours used were balanced within food versus  
195 no food stages for dietary contingencies. Technical and sample size reasons prevented all  
196 colour combinations being balanced. Therefore, only stages at which initial colour biases may  
197 have affected learning were balanced. It was assumed any initial biases would have been  
198 modified by experience by the experimental versus control diet stage. The colours used were  
199 as follows:

200 CVC group: 1) Control diet versus no food task: green versus yellow (balanced for diet  
201 option: colour; hereafter B); 2) CAP diet versus no food task (B): purple versus orange; 3)  
202 CAP diet versus Control diet: orange (CAP diet) versus green (Control diet) OR purple (CAP  
203 diet) versus yellow (Control diet).

204 CVF group: 1) Control diet versus no food task: red versus black (B); 2) FIBRE diet versus  
205 no food task: white versus blue (B); 3) FIBRE diet versus Control diet: red (FIBRE diet)  
206 versus blue (Control diet) OR black (FIBRE diet) versus white (Control diet).

#### 207 2.5.3.3. T-maze training protocol

208 The general procedure for each trial was as follows: at the start of the day, the T-maze was  
209 placed in the runway between the two parallel rows of 'terminal' testing pens. The distal exit  
210 holes at the end of the T-maze arms were lined up with the guillotine hatchways (which were  
211 secured open) of the end two pens. The appropriate coloured inserts were attached to the  
212 appropriate arms of the T-maze. The bird allocated to these pens was collected from its home  
213 pen, placed in the start box and held for 30 seconds. The Perspex door was then lifted and the  
214 bird was allowed to walk through the runway apparatus and exit into either terminal pen. The  
215 bird was then closed into this pen. How long the bird was held in this pen depended on the  
216 task and is described below in the food versus no food task and experimental diet versus  
217 control diet section. Once the trial was completed, the bird remained in the terminal pen until  
218 it was re-tested (circa 90 minutes). To allow further birds to be tested during this period, the  
219 T-maze was then moved along the walkway to line up with the next set of terminal pens and  
220 the next bird tested. This procedure was repeated until all six consecutive pairs of terminal  
221 pens had been used. The same procedure then took place in the second walkway and the  
222 second set of parallel pairs of 'terminal' pens. All the birds from one group were trialled in  
223 the same walkway/set of pens (i.e. CVC birds occupied the pens in walkway one and CVF  
224 birds the pens in walkway two).

#### 225 2.5.3.4. Food versus no food task

226 Birds were initially given 35 trials (seven consecutive days) per diet (phases 1 and 2) to  
227 learn separately about the post-ingestion ingestion feedback effects of each diet, and to learn  
228 to associate a certain colour with each diet. In phase 1, half the birds were randomly allocated  
229 to be trained with the control diet vs. no food, while the remainder were trained with their

230 experimental diet (FIBRE or CAP) vs. no food. In phase 2 each bird then learnt the other  
231 contingency. Birds were trained in a discrimination task between colour X = food and colour  
232 Y = no food. If the bird made the wrong choice (i.e. it selected the pen containing no food) it  
233 was held in its chosen pen for 1 minute before the hatchway was raised and the bird allowed  
234 to re-enter the T-maze. The hatchway was then closed behind the bird, in effect forcing it to  
235 choose the correct (food rewarded) pen. Once it had entered this pen the trapdoor was closed  
236 behind it and the bird was allowed to consume the food. The bird then remained in this pen  
237 for approximately 90 minutes (until the next trial).

238 Immediately after phases 1 and 2 had been completed, the birds were given twenty  
239 'refresher' trials per diet (experimental diet versus no food and control diet versus no food  
240 tasks) to remind them of the post-ingestion effects of each diet and the colour-diet type  
241 association (phases 3 and 4). The diet the birds experienced in phase 1 was offered to them in  
242 phase 3 and the diet offered to them in phase 2 was offered to them in phase 4. This re-  
243 presenting of the diet-colour combinations ensured that the birds had retained the information  
244 learnt after a period of time not exposed to the diet-colour combination as we were concerned  
245 the association might have extinguished without regular reinforcement and this would affect  
246 any preference seen.

247 The first fifteen of these trials for each diet were consecutive (i.e. phase 3 was five trials per  
248 day for 3 days of one diet then the same procedure was followed for phase 4). The last five  
249 trials of each phase were organised (five per day over 2 days) such that the task was alternated  
250 between the experimental diet versus no food task and the control diet versus no food task  
251 (five trials per diet spread over 2 days). These final 2 day period data were analysed as though  
252 it was a fourth day of phases 3 and 4.

#### 253 2.5.3.5. Experimental diet versus control diet (phase 5)

254 After the food versus no food training had been completed, birds were given ten trials (over  
255 2 days) in which they could choose between a portion of control diet and a portion of



256 experimental diet. The procedure was as described above for the food/no food task but with  
257 one exception: there was no ‘wrong choice’ and birds remained in the pen they selected first.  
258 This phase had been planned to last 35 trials (7 days) but ended early after ten trials due to the  
259 unexpected behaviour of the birds (see Results).

#### 260 2.5.4. Statistical analysis

261 Unless otherwise stated in the results section(s), all statistical tests were performed using  
262 Genstat (Version 11.1, VSN Ltd., 2008).

##### 263 2.5.4.1. Two-pan choice test – initial dietary preference test

264 Only the first 10 min of feed intake was analysed due to rapid consumption. After this point,  
265 for all birds, total (both diets) intake approached 100% rendering preference quantification  
266 meaningless.

267 Data were initially expressed as intake of each diet as a proportion of total intake during  
268 each session. However, the transformed data (arc-sine transformation), were neither normal  
269 (Shapiro-Wilk normality test) nor homogeneous (Barlett’s Test for variance homogeneity).  
270 Thus the proportional intakes were analysed non-parametrically using the Kruskal-Wallis  
271 (within treatment between day comparisons) and Wilcoxon Matched Pairs test (comparisons  
272 between average daily consumption of each diet by each bird).

##### 273 2.5.4.2. T-maze choice test experiment

274 For all phases of the T-maze choice experiment (including subsequent modification to the  
275 study design), a Generalised Linear Mixed Model (GLMM) was used to investigate the  
276 following fixed effects: treatments, phases, days, colour-combinations (random effect:  
277 bird/trial) and bird (random effect: side) and to generate logit-transformed predicted means  
278 (group daily and overall mean). The response variate used for all analyses was ‘diet option  
279 chosen’. Where the GLMM could not model the data using the F-ratio (F) the Wald statistic  
280 (W) is reported. Post-hoc group analyses of differences from 0.5 (i.e. no preference shown)

281 were manually calculated using  $\chi^2$  to compare for differences from 0 at 1 degree of freedom  
282 using a Chi-squared ( $\chi^2$ ) – distribution table (Petrie & Watson, 1999). The test statistic (T)  
283 used for this was:

284 
$$T = (\text{predicted mean} / \text{S.E. of the predicted mean})^2$$

285 Individual bird differences from 0.5 were calculated using binomial probability distribution  
286 tables.

287 Side bias severity scores were calculated by blocking data into groups of 10 consecutive  
288 trials. The blocks of data used were: phases 1 and 2 (first 10 trials), phases 3 and 4 (last 10  
289 trials) and phase 5 (all ten trials). From these data an individual bird score was calculated.  
290 10/10 and 0/10 represented 100% preference for the right and left side respectively. To  
291 convert to a severity score (independent of preferred side), each bird's ten-trial score was  
292 reassigned a new 'side bias severity' score (0 – 5, 5 being the severest bias possible):

<b>Original score</b>	0	1	2	3	4	5	6	7	8	9	10
<b>New score</b>	5	4	3	2	1	0	1	2	3	4	5

293

294 This data were analysed using the Kruskal-Wallis test. Pair-wise post-hoc testing was  
295 performed using the Mann-Whitney U Test. The pairs tested were phase 5 versus, phase 1  
296 (first 10 trials), phase 2 (first 10 trials), phase 3 (last 10 trials) and phase 4 (last 10 trials).

#### 297 2.5.4.3. Modifications

298 Unless otherwise stated within the results section, data were analysed as in section 2.5.4.2.

#### 299 2.5.4.4. Ethical considerations

300 This study was carried out under the Animals (Scientific Procedures) Act 1986 and approved  
301 by the Scottish Agricultural College's and Roslin Institute's ethics committees. The Home  
302 Office Code of Recommendations for the housing of poultry was met or exceeded at all times.

303 Birds were euthanised by an approved Schedule One method (barbiturate anaesthetic  
304 overdose).

### 305 3. Results (1)

#### 306 3.1. Two-pan choice test – initial dietary preference experiment

##### 307 3.1.1. Control diet versus CAP diet (CVC)

308 Overall, the birds showed a preference for the control diet ( $W = 9, n = 12, P = 0.016$ ), based  
309 on the individual mean intake of 12 birds over 10 occasions (5 days observations). The mean  
310 % intake of the control diet by the birds was 57%. However, there was considerable variation  
311 within-bird between the different tests (mean standard deviation of within-bird variation in  
312 control diet consumed as a proportion of total intake in a session = 0.23) (see: figure 2). There  
313 was no significant effect of bird, day or session (AM/PM).

##### 314 3.1.2. Control diet versus Fibre diet (CVF)

315 Overall, the birds did not express a preference for either diet ( $W = 31.0, n = 12, P = 0.569$ ),  
316 based on the mean intake of 12 birds over 10 occasions (5 days observations). The mean %  
317 intake of the control diet by the birds was 49%. However, there was considerable variation  
318 within-bird between the different tests (mean standard deviation of within-bird variation in  
319 control diet consumed as a proportion of total intake in a session = CVF: 0.23) (see: figure 2).  
320 There was no significant effect of bird, day or session (AM/PM).

321 **FIGURE TWO SHOULD GO HERE**

#### 322 3.2. Food versus no food discrimination trials

##### 323 3.2.1. Initial 'learning' trials (phases 1 and 2)

324 Overall, analysed at the group-level, birds in both groups showed a preference for the  
325 colour associated with food in the food versus no food trials (CVC treatment group: phase 1:  
326  $\chi^2 = 21.19, d.f. = 1, p < 0.001$ ; phase 2:  $\chi^2 = 43.54, d.f. = 1, p < 0.001$ ; CVF treatment group:  
327 phase 1:  $\chi^2 = 17.89, d.f. = 1, p < 0.001$ ; phase 2:  $\chi^2 = 48.22, d.f. = 1, p < 0.001$ ). There was a

328 significant effect of day ( $F_{(6,823.0)} = 15.89, P < 0.001$ ) with birds picking the food option  
329 significantly more often than the no food option during the last few days of phases 1 and 2  
330 indicating that they had learnt to associate the colour with food (Figures 3 and 4). There was  
331 also an effect of phase ( $W_{(1,7.27)} = 7.27 P = 0.007$ ) with birds showing a stronger preference  
332 for the food over the no food option in phase 2, indicating that they found the task easier to  
333 learn the second time. There were no other significant effects or interactions (including diet  
334 option offered). In phase 1, 10/12 birds (5/6 in each treatment group individually performed  
335 better than chance in the last 20 trials ( $\geq 15/20$  choices for the food option  $p \leq 0.041$ ). The  
336 remaining birds selected the correct option 14/20 times. In phase 2, all birds met this criterion  
337 over the last four days.

### 338 3.2.2. 'Refresher' trials (phases 3 and 4)

339 Overall, both treatment groups showed a preference for the colour associated with food in  
340 the food versus no food refresher trials (CVC treatment group: phase 3:  $\chi^2 = 36.19, d.f. = 1, P$   
341  $< 0.001$ ; phase 4: CVC:  $\chi^2 = 43.13, d.f. = 1, P < 0.001$ ; CVF treatment group: phase 3:  $\chi^2$   
342  $36.2, d.f. = 1, P < 0.001$ ; phase 4:  $\chi^2 = 49.67, d.f. = 1, P < 0.001$ ), indicating that they had  
343 retained both colour-food/no food associations after a period of 4 – 7 days of no exposure to  
344 each combination (whilst the other combination association was being trained/refreshed).

345 There was a phase effect ( $F_{(1,460.0)} = 6.08, P = 0.014$ ), with birds in both treatment groups  
346 performing better in phase 4 than in phase 3. However, all birds individually performed better  
347 than chance in each of the 'refresher' phases ( $\geq 15/20$  choices for the food option,  $p \leq 0.041$ ).

348 An effect of day was also apparent ( $F_{(3,460.0)} = 3.02, P = 0.030$ ) with birds increasingly  
349 picking the colour-food option over time in phase 3. However, irrespective of phase, both  
350 treatment groups showed a significant preference for this option shown from day 1 (Figure 3).  
351 There were no other significant effects or interactions (including diet option offered).

352 FIGURE THREE SHOULD GO ABOUT HERE

353 3.3. Experimental diet versus control diet (phase 5)

354 At the group level no diet preference was observed (CVC:  $X^2 = 0.04$ , d.f. = 1,  $P > 0.1$ ; CVF:  
355  $X^2 = 1.8$ , d.f. = 1,  $P > 0.1$ ) and only one bird showed a significant diet preference (9 out of 10  
356 choices were for the control diet option,  $p < 0.05$ ). However, birds in both treatment groups  
357 showed side biases with 3 out of 6 birds in each treatment group showing a significant side  
358 bias (9 out of 10 choices for a specific side,  $p < 0.05$ ) and a further 4 birds selecting a specific  
359 side 8 out of 10 sides. There were no other significant effects or interactions on either diet or  
360 side preferences. Therefore, data from both groups was combined in an analysis of side biases  
361 observed.

362 A comparison between any potential side biases observed in Phase 1 (1<sup>st</sup> ten trials), phase  
363 2 (1<sup>st</sup> ten trials), phase 3 (last ten trials), phase 4 (last ten trials) and phase 5 (control versus  
364 experimental diet; all ten trials) indicated that there was a significant phase effect ( $H = 26.59$ ,  
365 d.f. = 4,  $P < 0.001$ ). Individual birds picked the same side pen on repeated trials significantly  
366 more often in phase 5 compared to any other preceding phase: phase 1 (1<sup>st</sup> ten trials),  $U =$   
367  $17.0$ ,  $n = 12$ ,  $P < 0.001$ ; phase 2 (1<sup>st</sup> ten trials),  $U = 26.0$ ,  $n = 12$ ,  $P = 0.003$ ; phase 3 (last ten  
368 trials),  $U = 10.0$ ,  $n = 12$ ,  $P < 0.001$ ; phase 4 (last ten trials),  $U = 4.0$ ,  $n = 12$ ,  $P < 0.001$ .

369 4. Discussion (1)

370 4.1. Initial dietary preferences

371 The results indicated that CVC birds showed a small preference for the control diet and  
372 CVF birds did not show a preference. This suggested the CAP diet was initially less liked  
373 than the FIBRE diet or the control diet. One possibility for the failure to show any or strong  
374 preferences is that the birds consumed almost all the entire total ration (control diet plus  
375 experimental diet) within 10 minutes and thus any preference was hidden. However, an  
376 analysis of the data (not reported here) in which any bird that consumed more than 75% of the  
377 total ration (CVC birds) or 60% of the ration (CVF birds) was excluded from the analysis for  
378 the feeding session in which the cut-off point was exceeded obtained the same direction of

379 preference (CVC group) or lack of preference (CVF birds) reported here with minimal, non-  
380 significant effects on strength or direction of any preference). Different ‘cut off’ points were  
381 selected for each treatment group in this alternative analysis due to the quantity of  
382 experimental diet being different between the two groups. However, the small quantities of  
383 food offered remained a serious limitation that potentially affected interpretation of the  
384 findings as the strength of any potential preference was artificially truncated. It was not  
385 possible to offer true *ad libitum* conditions due to this being a preparatory phase for the main  
386 experiment. With hindsight, one daily feeding session would have benefited data collection in  
387 view of the rapid feed consumption.

388       Within bird, the proportion of control diet consumed varied considerably between feeding  
389 opportunities. Anecdotal observations suggested that this was because birds stuck with the  
390 first bowl of food they encountered and stayed until most of that ration was consumed. This  
391 may reflect diet type indifference. However, it may also reflect hunger state. High  
392 motivational drive to rapidly consume any food found might initially have suppressed  
393 motivation to obtain a more favourable food source. At five weeks feed restriction is already  
394 severe: on day 35 birds reared conventionally weigh an average of 560g and are fed circa 44g  
395 / day. This is considerably less than an *ad libitum* fed broiler breeder would consume on day  
396 35 (average 159.8g/day consumed) or with an average bodyweight of 577g (average 93g/day  
397 consumed) (unpublished research by authors). This may have seriously impeded the  
398 exhibition of preferences.

399       In summary, the approach taken was not useful for evaluating sensory-led initial dietary  
400 preferences due to insufficient food quantities offered and the nature of the birds’ feeding  
401 behaviour.

#### 402           4.2.     Performance during choice test

403       Birds found it easy to learn a food/no food discrimination task and they were able to retain  
404 this information. However they then failed to show a diet preference in the control diet versus

405 experimental diet choice test. Side preferences more clearly explained bird performance than  
406 diet preference. It was unclear whether the development of side biases observed was a  
407 consequence of dietary option indifference, failure to associate diet type with colour, failure  
408 to transfer knowledge in the previous phases to the new, novel colour pairings or inability to  
409 distinguish between diets. To investigate these potential explanations the experimental design  
410 was modified.

411

## 412 5. Experimental modifications

### 413 5.1. Novel colour-pairing

#### 414 5.1.1. Hypothesis

415 It was hypothesised that if the birds could transfer knowledge learnt in previous colour-  
416 pairings to novel colour-pairings then they would immediately prefer the food-rewarded  
417 option.

#### 418 5.1.2. Method

419 Two novel colour pairs were created by switching the no food colours: the no food colour  
420 originally paired opposite the control diet colour was now paired opposite the experimental  
421 diet colour (and vice versa). The diet outcomes associated with each colour did not change.  
422 Birds were given 30 trials (6 days): ten per new colour pairing option and per Control versus  
423 Experimental diet option. Trials were blocked into groups of three. Each block contained one  
424 trial of each option. Within block trial order was randomised to reduce effects of current  
425 learning on performance (as opposed to choices reflecting previous learning).

### 426 5.2. Experimental diet versus control diet (2)

#### 427 5.2.1. Hypothesis

428 It was assumed that if birds primarily attended to the ‘no food’ colours (i.e. they avoided the  
429 ‘no food’ option rather than specifically attended to “X” colour is associated with “X” diet  
430 option and “Y” colour is associated with “Y” diet option) then removal of this option would

431 force attendance to the 'food' colours and result in discrimination between the two diet  
432 options (i.e. control diet and experimental diet). It was hypothesised that, in the continued  
433 absence of a 'no food' option birds would learn to associate colours with diet quality and  
434 would show a preference for the more favourable option.

#### 435 5.2.2. Methods

436 Birds were given 35 trials (7 days) of the control diet versus the experimental diet options.

### 437 5.3. Experimental diet versus control diet + 50%

#### 438 5.3.1. Hypothesis

439 It was hypothesised that if birds could learn to associate colours with differences in the  
440 properties (quality or quantity) then they would develop a preference for an option that  
441 provided more energy and nutrients.

#### 442 5.3.2. Methods

443 The control diet was increased by 50% to make it more attractive to hungry birds. Birds  
444 were given 55 trials (11 days) of the control diet versus the experimental diet options. Colours  
445 associated with each diet remained the same.

### 446 5.4. Two-pan choice test: experimental diet versus control diet

#### 447 5.4.1. Hypothesis

448 Sensory diet discrimination is essential otherwise no choice is possible irrespective of how  
449 nutritionally diverse two diets are (Forbes and Kyriazakis, 1995). Although this had been  
450 previously tested in the pre-sensory phase, the lack of preference shown by CVF birds and the  
451 small preference shown by CVC birds potentially suggested that they have difficulties in  
452 discriminating between diets. Thus, despite how unlikely this may be, it was necessary to  
453 establish that the birds could distinguish between diets *per se*. It was hypothesised that, if the  
454 birds could discriminate between the two diets offered then they would prefer the control diet  
455 under simultaneous presentation with ad libitum access to both feeds.

#### 456 5.4.2. Methods



457 Birds were tested on the final study day then humanely euthanized. Experimental apparatus  
458 was set up as in section 2.4.1. Each bowl was filled approximately  $\frac{3}{4}$  full with either  
459 experimental or control diet which had been weighed. Within group, diet presentation was  
460 balanced for side (control diet initially on left side for 50% of birds). At 0 min birds were  
461 placed into individual pens and allowed to freely consume from both bowls. At 10 min food  
462 was removed, weighed, replenished and returned to the pens (switched to the opposite side).  
463 At 20 min the procedure was repeated. At 30 min the food was removed, weighed and  
464 discarded.

## 465 6. Results (2)

### 466 6.1. Novel colour pairing

467 The results indicated that whatever the birds had learnt about the original training  
468 conditions they were able to transfer into the novel testing situation (CVF:  $\chi^2 = 36.20$ , d.f. = 1,  
469  $P < 0.001$ ; CVC:  $\chi^2 = 46.49$ , d.f. = 1,  $P < 0.001$ ; Figure 4). There were no other significant  
470 effects or interactions. Individually, all birds achieved  $\geq 15/20$  ( $p \leq 0.041$ ) choices for the  
471 colour associated with food.

472

473 FIGURE FOUR GOES ABOUT HERE

474

### 475 6.2. Experimental diet versus control diet (2)

#### 476 6.2.1. Food preferences

477 As a group, birds did not exhibit a preference for either diet, either across all trials (CVC:  $\chi^2$   
478 = 0.35,  $P > 0.1$ ; CVF:  $\chi^2 = 0.23$ ,  $P > 0.1$ ) or across days (Figure 5). There was no effect of  
479 treatment or day and no interaction between day and treatment. However, there was a highly  
480 significant effect of bird on the choices made ( $W_{(11,3,31)} = 36.41$ ,  $P < 0.001$ ). Post-hoc testing  
481 indicated that two CVC birds and two CVF birds showed a significant preference for the  
482 control diet over the 35 trials. Four CVC birds and four CVF birds failed to show a diet  
483 preference.

484

485 FIGURE FIVE GOES ABOUT HERE

486

487 6.2.2. Side biases

488 None of the birds that showed a diet preference showed a side bias. Of the eight birds that  
489 did not show a diet preference, seven showed a significant ( $P < 0.05$ ) side bias; the remaining  
490 bird tended ( $P = 0.09$ ) to prefer one side over the 35 trials.

491 However, at the group level, there was no difference between the severity of side biases  
492 demonstrated in phase five (control versus experimental diet) and those exhibited in either the  
493 first or last ten trials of this phase (control versus experimental diet;  $H = 2.136$ , d.f. 2,  $P =$   
494 0.328).

495 6.3. Experimental diet versus control diet + 50%

496 6.3.1. Food preferences

497 Although neither treatment group showed an overall preference for either the experimental  
498 diet or the '50% extra' control diet (CVC:  $\chi^2 = 0.01$ , d.f. = 1,  $P > 0.1$ ; CVF:  $\chi^2 = 2.25$ , d.f. = 1,  
499  $P > 0.1$ ), there were several days on which the CVC group selected the control diet  
500 significantly more often (see figure six). However, the 'performance' of both groups was  
501 sufficiently similar that significant differences between groups were not found either overall  
502 or by day, and there was no interaction between treatment group and day.

503

504 FIGURE SIX GOES ABOUT HERE

505

506 However, there was a highly significant effect of bird on the choices made ( $W_{(11,4,50)} =$   
507 49.50, d.f. = 1,  $P < 0.001$ ). Post-hoc testing indicated that three CVC birds and two CVF birds  
508 showed a highly significant preference for the control diet over 55 trials. A further CVC bird  
509 had a tendency to select the control diet and one CVF bird had a tendency to pick the fibre  
510 diet. The remaining five birds failed to show a diet preference, either over all 55 trials or over  
511 the last 15 trials.

512                   6.3.2. Side biases

513       Side biases remained prevalent. All birds that failed to show a significant diet preference  
514 (plus one CVC that did) demonstrated a side bias. Eight birds showed either a highly  
515 significant ( $n = 4$ ,  $P < 0.001$ ) or significant ( $n = 2$ ,  $P \leq 0.014$ ) side preference or had a  
516 tendency to pick one side more ( $n = 2$ ,  $P = 0.058$ ) over 55 trials.

517

518                   6.4. Two-pan choice test: Experimental diet versus control diet

519       Overall, there was a highly significant effect of diet ( $T = 1.0$ , d.f. = 11,  $p < 0.001$ ), with 11  
520 out of 12 birds preferring the control diet. This demonstrated very clearly that the birds were  
521 able to distinguish between the two diets (Figure 7).

522

523       FIGURE SEVEN SHOULD GO ABOUT HERE

524

525       Within the CVF group, there was a significant preference for the control diet ( $T = 0.0$ , d.f.  
526 5,  $p = 0.031$ ) with all six birds preferring the control diet. Within the CVC group, there was a  
527 tendency to prefer the control diet ( $T = 1.0$ , d.f. 5,  $p = 0.062$ ). However, this was probably due  
528 to the small sample size and lack of statistical test sensitivity (the non-parametric Wilcoxon  
529 Matched Pairs test was used for all three analyses), as five out of six CVC birds showed a  
530 strong preference for the control diet.

531

532                   7. General discussion

533                   7.1. Modifications

534       Clearly bird failure was not due to inability to transfer learnt information to solving a novel  
535 task or to distinguish between diets. Therefore, the lack of diet preference observed under T-  
536 maze choice test conditions seemed due to difficulties associating diet quality and quantity  
537 differences with different colour maze arms. Reasons for this are discussed below.

538                   7.2. Observed diet preferences

539 The birds strongly preferred the control diet under *ad libitum* conditions. High energy-  
540 density diets are often highly preferred (Brunstrom and Mitchell, 2007; Bouvarel *et al.*, 2009;  
541 Bolles *et al.*, 1981). Utilising a similar two-pan, *ad libitum* access, choice test, Guillemet, *et*  
542 *al.*, (2007) found gestating sows (highly food motivated) also prefer high quality nutrient  
543 dense feed to quality-adjusted, high fibre feed. Preference for nutrient-dense diets makes  
544 evolutionary sense: animals need to balance feed intake against other needs (for example,  
545 reproduction, predation avoidance, etc) (Lieberman, 2006; Illius *et al.*, 2002). Therefore, the  
546 direction of the preference observed was unsurprising.

547 Where significant preferences developed under closed economy, feed restricted conditions  
548 (prior to increasing control diet quantity) as they did for two CVC and two CVF birds, these  
549 preferences were also for the high quality, nutrient dense control diet. D'Eath, *et al.*, (2009)  
550 suggests animals' preference for high quality feed over low quality feed might disappear  
551 under restricted feed conditions if the low quality feed confers improved satiety. Our results  
552 did not support this. **However**, we cannot rule out whether this was due to the experimental  
553 diets not having increased satiating effects (therefore not addressing the point) or impulsivity  
554 influencing choice by biasing any preferences towards the most rapidly consumable diet.  
555 Abeyesinghe, *et al.* (2005) found that chickens showed self-control only between a small  
556 immediate reward and a delayed (much) larger reward. This implied a need for the  
557 experimental diet to be much more rewarding if it is to be preferred. Although there was no  
558 time delay imposed on diet access, the experimental diets would take longer to consume  
559 compared to the control due to diet bulkiness (FIBRE) or additive fineness (CAP) (intake rate  
560 not measured). Anecdotal observation **(unfortunately this was not formally measured)**  
561 indicated that the latency to consume either experimental ration fully rapidly decreased.  
562 However, this reduction may have been concurrent with a gradual decrease in satiating  
563 capacity due to physiological adaptation to the additional dietary components (Tolkamp *et al.*,  
564 2005) further reducing its additional 'rewarding' features over the control diet. **Alternatively,**  
565 **it is possible that the use of a schedule in which the birds alternated between the control and**

566 experimental diets created a situation in which, even if the experimental diets had increased  
567 satiating effects, because the birds were not maintained continuously on the experimental diet,  
568 the full satiating effects of these quality-adjusted diets were not achieved. Thus, the birds  
569 tested may not have been in the same physiological and / or affective state as birds reared  
570 continuously under conditions of qualitative feed restriction and this may have impacted upon  
571 both their ability to learn the task and / or to express any preference learnt.

572 In addition, group feeding species (including chickens) already eat faster than solitary  
573 feeders (Sunday, 1981, quoted in (Ackroff, 2002) and chickens have been shown to have a  
574 greater motivational drive to feed fast under chronic than acute feed restriction (Savory, *et al.*,  
575 1993). Thus, the combined effect of species-specific characteristics and strong motivational  
576 drive may increase preference for rapidly consumable high quality feed, irrespective of  
577 possible later differences in diet-induced satiation. However, the design of the study may also  
578 have affected the presence or absence of preferences observed as the birds may never have  
579 experienced the degree of satiety that being reared entirely on a qualitatively restricted diet  
580 may offer. It remains a problem for choice test methodologies of this nature: the birds are  
581 inevitably reared, trained and tested under conditions that are not similar to commercial  
582 environments. However, as the current methodologies utilising environments close to those  
583 experienced under commercial conditions also fail to provide convincing evidence of the  
584 benefits or otherwise of qualitatively restricted diets these alternative approaches should be  
585 explored.

### 586 7.3. Methodological issues

587 A long inter-trial interval (ITI) ensured birds experienced the ‘satiating’ effects of their  
588 choice through the mechanism of post-ingestion feedback. Matthews and Temple (1979),  
589 used an operant choice test to allow dairy cows to access small quantities (time restricted  
590 access ~ 5 seconds, ITI variable interval 60 – 300 seconds) of either concentrate or hay. The  
591 authors claimed this allowed diet preference quantification without the confounding variable

592 of post-ingestion effects. However, this can be a limitation. Post-ingestion feedback shapes  
593 longer-term diet preferences (Forbes, 1998; Kyriazakis *et al.*, 1999). Thus, we wanted a  
594 longer ITI with larger portions/trial.

595 However, it is possible the ITI was too long (90 min) for colour-diet information retention.  
596 Our birds easily learnt the food/no food task. Direct comparisons between speed of learning  
597 this task and the quality/quantity discrimination tasks are not methodologically possible.  
598 However, rats performed better with spaced trials than with massed trials (Sarason *et al.*,  
599 1956) but the ITI used in that case was only 12 minutes long. Pennington & Thompson  
600 (1958) found the number of trials needed for rats to reach the criterion increased with ITI  
601 length (ITI lengths compared: 40 min – 24 hr). However, other studies found a positive,  
602 negative or no effect of ITI on learning (D'Amato, 1960).

603 Failure to learn could also be attributed to decreased differential in terms of comparative  
604 option payoffs which increased task complexity. Rats learnt food-no food discrimination tasks  
605 more quickly than food quantity discrimination tasks (Clayton, 1964). Further, rat (Clayton,  
606 1964; Hill and Spear, 1963) and dolphin (Mitchell *et al.*, 1985b) acquisition rates are a  
607 function of the contrast between two reward quantities. We could not find any papers  
608 investigating feed quality effects on acquisition rate in similar choice test apparatus.  
609 However, non-feed restricted layer hens quickly associated diets with colours in a heavy-  
610 metal feed contamination versus no contaminated discrimination task (Phillips and Strojjan,  
611 2007). Although we cannot discard methodological reasons causing or contributing to the  
612 failure of most birds to learn the food quality and quantity discrimination tasks, the success of  
613 some birds indicated the task was potentially learnable. Thus, we were led to consider the  
614 internal physiological and affective state of the birds as a potential causal factor.

#### 615 7.4. Hunger and stress

616 Hunger-stress may have decreased the birds' learning ability. Although motivation to gain  
617 feed increases with degree of feed restriction (Savory *et al.*, 1993; Bokkers *et al.*, 2004),

618 hunger is also a stressor (Mendl, 1999). The Yerkes-Dodson model (Yerkes and Dodson,  
619 1908) suggests there is a bell curve effect to arousal with an optimal level of arousal for  
620 effective learning. Although the model is simplistic (Mendl, 1999) a useful basic interpretive  
621 framework is provided by it. The birds' success at learning the food-no food tasks but failure  
622 to learn the food quantity/quality choice tasks corresponded with increasing severity of feed  
623 restriction. More complex tasks have a lower optimal arousal level (Yerkes and Dodson,  
624 1908). Stress can reinforce inflexible, habitual learning (Mitchell *et al.*, 1985a) leading to  
625 poorer performance. Therefore, poor learning may have been the combined effect of being too  
626 hungry and the dietary option contrast being too small.

#### 627 7.5. Side biases and stress

628 Although side biases may be an artefact of study design as chickens show low levels of  
629 spontaneous alternation in T-mazes (Haskell *et al.*, 1998) we found that side biases increased  
630 with the change from the food/no food to food quality discrimination tasks. Feed restriction  
631 severity was also increasing throughout this study. Side biases are more prevalent in hunger  
632 stressed starlings (Talling *et al.*, 2002) and electric-shock stressed rats (Rodriguez *et al.*,  
633 1992). These preferences can manifest as increased perseverance (Rodriguez *et al.*, 1992).  
634 Further, feed-restricted pigs in a food-no food T-maze task showed side biases even when  
635 they could see food in the non-selected pen (Rodriguez *et al.*, 1992). Reducing pig arousal by  
636 reducing time in the start box improved performance (pigs picking food option). These  
637 findings suggest species-specific tendencies reinforced by the effects of stress may have  
638 affected T-maze performance in our study.

#### 639 8. Conclusions

640 In conclusion, the selected T-maze task was not useful in investigating the feed preferences  
641 of chronically feed restricted broiler breeders. Although where birds did learn the task they  
642 preferred a small quantity of high quality feed to a quality-adjusted diet, the small number  
643 that did so limit any firm conclusions. Nevertheless, should a larger sample size replicate this

644 preference, this would be an interesting avenue for further investigation. However, preference  
645 exploration is based on the implicit assumption that cognitive capacity to learn and exhibit a  
646 preference is not undermined by chronic hunger stress. This suggests that the impact of  
647 chronic hunger-stress on broiler breeder learning should be studied first in further  
648 investigative research focussing on feed restricted broiler breeder dietary preferences.

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792

# Figure 1

Figure 1: Experimental set-up utilised in the T-maze choice test experiment. Additional terminal testing pens are omitted for clarity.

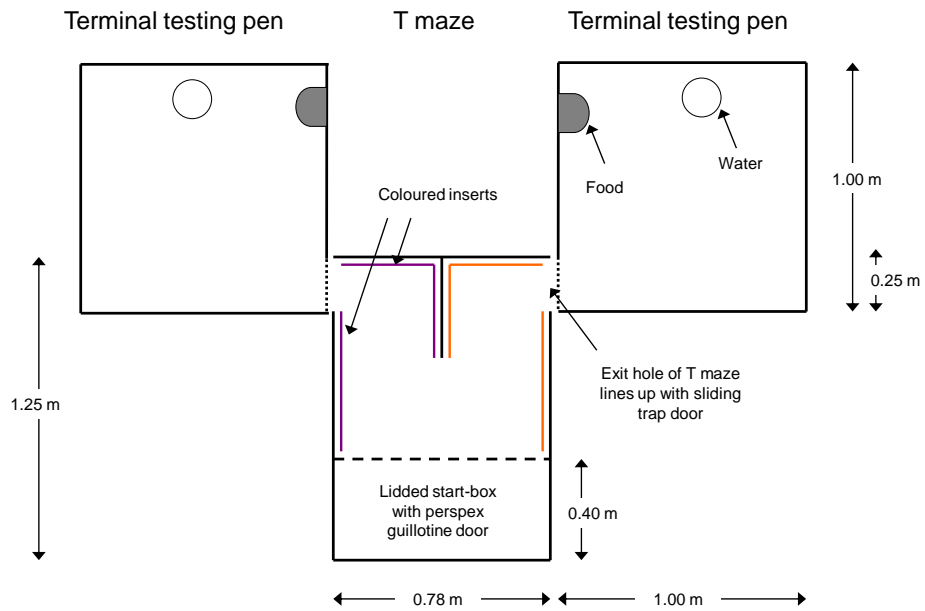


Figure 2

Figure 2: Mean individual bird intake of the control diet during the initial two-pan choice test expressed as a proportion of total diet (experimental + control) consumed within 10 minutes. The errors bars indicate the standard deviation for the within-bird variation across data points. Data was collected on 10 separate occasions (2 sessions per day for 5 consecutive days).

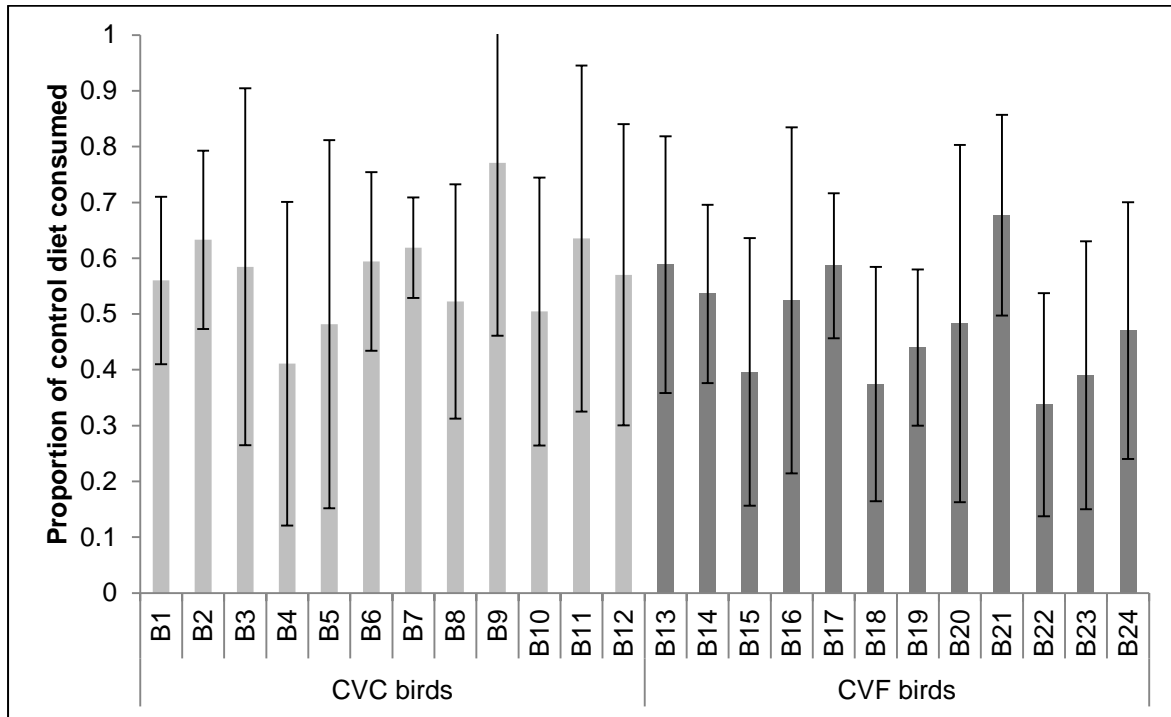
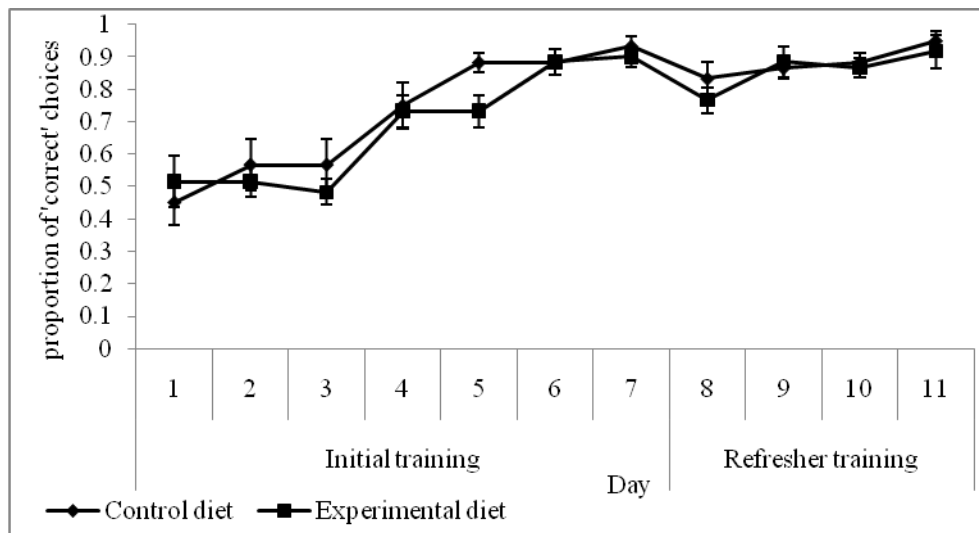


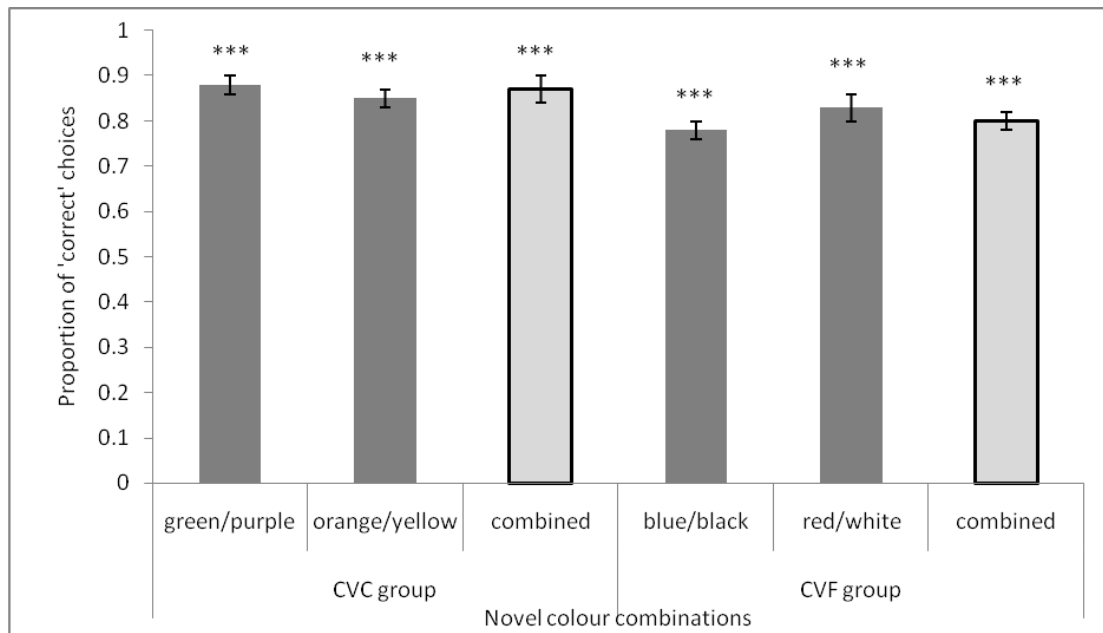
Figure 3: Food versus no food trials: proportion of ‘correct’ choices by diet (experimental or control option) × trial day. Data for both treatment groups has been combined, as there was no significant difference between treatment groups in terms of learning the food versus no food task (irrespective of diet option). Hence, experimental diet refers to both the CAP diet and the FIBRE diet. A preference for the food option ( $X = 4.31$ , d.f. 1,  $p < 0.05$ ) was observed on days 5, 6, 7, 8, 9, 10, 11 (control diet) and days 6, 7, 9, 10, 11 (experimental diet). Error bars = s.e.m. Figure legend: closed diamond = control diet; closed square = experimental diet.

† Although a continuous line is drawn through days 1 – 11 to aid clarity, the reader is reminded that birds had a 4 or 7 day break between day 7 and day 8 to allow the other diet – colour combination training (initial and / or refresher) to occur. Day 11 (phase three and four) is a composite day and actually took place over two days as the last five trials for each were alternated by trial.



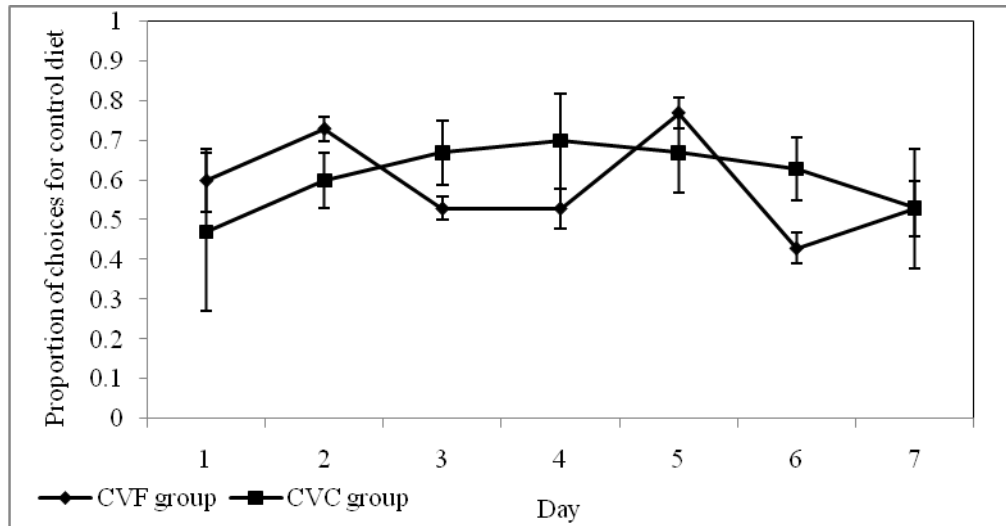
**Figure 4**

Figure 4: Effect of novel colour combination on proportion of 'correct' (food –rewarded) options (see: section 6.1). Combined for each treatment represents the combined result of both colour-combinations within that treatment. \*\*\* =  $P < 0.001$ . The error bars represent the S.E.M. associated with each combination.



**Figure 5**

Figure 5: Daily proportion of choices for either the control diet or the experimental diet by each treatment group (see: section 6.2). 1 = 100% preference for control diet, 0 = 100% preference for experimental diet. There were no days on which a significant group preference for one of the diets (i.e. a significant difference from 0.5 choices for control diet) was shown. Error bars indicate the S.E.M. Figure legend: Closed diamond = CVF group; closed square = CVC group.





**Figure 6**

Figure 6: Proportion of choices for the control diet (50% extra) option each day by treatment group. There were no days on which the CVF group showed a significant preference. There were five days (day 1, 3, 4, 6, 9) on which the preference for the control diet was significant ( $p < 0.05$ ) for the CVC group. Error bars indicate the S.E.M. Figure legend: Closed diamond = CVF group; closed square = CVC group.

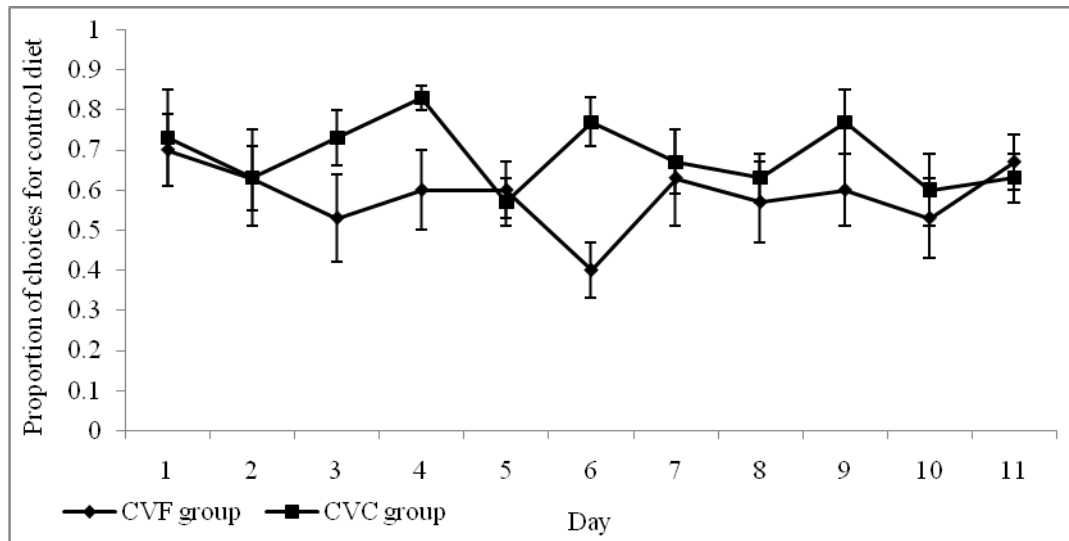


Figure 7: Mean consumption (grams) over 30 minutes over the experimental diet, control diet and total intake of both diets combined. The error bars represent the S.E.M..

