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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: 21st 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 Tolkamp 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

*Revision Note

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 rational 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).

*Manuscript

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.

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

1. Introduction

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

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

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

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

Choice tests are a novel way to navigate round this interpretive difficulty (D'Eath e*t al.*, 2009). Choice tests are widely used in evaluating animal welfare and assume an animal's preferred option would lead to enhanced welfare. Dawkins (2004) claims only two questions need answering when evaluating an animal's welfare: Is it healthy? Does it have what it wants? Healthy broiler breeders can be produced on a typical quantitative restriction diet or on a QDR (Tolkamp *et al*., 2005). Therefore, the remaining question is: do feed restricted broiler breeders prefer this feed restriction to be quantitative or qualitative?

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

2. Materials and methods

2.1. Subjects

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

2.2. Housing & husbandry

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

2.3. Nutrition & feeding

2.3.1. Growth curves

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

2.3.2. Starter diet and protocol

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

2.3.3. Experimental diet and protocol

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

Diet rations were designed to ensure equivalent growth, based on Tolkamp *et al*., (2005) who found that the quantity of basal feed (commercial feed minus CAP and OH) consumed ad lib by their QDR birds was similar to birds fed the commercial feed restricted ration. Initial dietary preferences were also investigated as initial dietary preferences are modifiable by post-ingestion feedback (Kyriazakis *et al*., 1999; Forbes, 1998; Provenza, 1995). Quantities of the compounds added were less than in Tolkamp, *et al* (2005) . This reflected previous unpublished findings by the authors that indicated that gradual adjustment to QDR may mean insufficient energy consumption initially if compound inclusion levels are high. Broiler breeders are sensitive to restriction severity (Savory *et al.*, 1993; Bokkers and Koene, 2004; Bokkers *et al.*, 2004), thus, we assumed, should prefer an increase in satiety, even if that satiety is not complete.

2.4. Experimental apparatus

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

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

2.4.2. T-maze choice test experiment

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

FIGURE 1 SHOULD GO ABOUT HERE

2.5. Training and testing

2.5.1. Handling and socialisation

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

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

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

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

Birds were given equal exposure to both the control and experimental diets. Both diets were offered simultaneously (each portion equalled 1/4 of total daily feed provided) with two feeding opportunities/day (09:00 h and 13:00 h) for 7 days. The rations offered at each feeding opportunity over this period were: control diet: 11g; CAP diet 11.3g and FIBRE diet: 15.7g. Individual feed intake was measured twice daily for the first 5 days. The food removed, weighed, and returned after 10, 20, 30, 40, 50, 60, 120, 180 and 210 min. Food left after 240 min was removed, weighed and discarded. Diet was balanced (within and between birds) for pen side and randomly switched sides between feeding opportunities.

2.5.3. T – maze choice test experiment

2.5.3.1. General testing protocol

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

2.5.3.2. Dietary contingencies associated with colours

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

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

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

2.5.3.3. T-maze training protocol

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

2.5.3.4. Food versus no food task

Birds were initially given 35 trials (seven consecutive days) per diet (phases 1 and 2) to learn separately about the post-ingestion ingestion feedback effects of each diet, and to learn to associate a certain colour with each diet. In phase 1, half the birds were randomly allocated to be trained with the control diet vs. no food, while the remainder were trained with their experimental diet (FIBRE or CAP) vs. no food. In phase 2 each bird then learnt the other contingency. Birds were trained in a discrimination task between colour X = food and colour Y = no food. If the bird made the wrong choice (i.e. it selected the pen containing no food) it was held in its chosen pen for 1 minute before the hatchway was raised and the bird allowed to re-enter the T-maze. The hatchway was then closed behind the bird, in effect forcing it to choose the correct (food rewarded) pen. Once it had entered this pen the trapdoor was closed behind it and the bird was allowed to consume the food. The bird then remained in this pen for approximately 90 minutes (until the next trial).

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

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

2.5.3.5. Experimental diet versus control diet (phase 5)

After the food versus no food training had been completed, birds were given ten trials (over 2 days) in which they could choose between a portion of control diet and a portion of experimental diet. The procedure was as described above for the food/no food task but with one exception: there was no 'wrong choice' and birds remained in the pen they selected first. This phase had been planned to last 35 trials (7 days) but ended early after ten trials due to the unexpected behaviour of the birds (see Results).

2.5.4. Statistical analysis

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

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

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

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

2.5.4.2. T-maze choice test experiment

For all phases of the T-maze choice experiment (including subsequent modification to the study design), a Generalised Linear Mixed Model (GLMM) was used to investigate the following fixed effects: treatments, phases, days, colour-combinations (random effect: bird/trial) and bird (random effect: side) and to generate logit-transformed predicted means (group daily and overall mean). The response variate used for all analyses was 'diet option chosen'. Where the GLMM could not model the data using the F-ratio (F) the Wald statistic (W) is reported. Post-hoc group analyses of differences from 0.5 (i.e. no preference shown) were manually calculated using χ^2 to compare for differences from 0 at 1 degree of freedom using a Chi-squared (χ^2) – distribution table (Petrie & Watson, 1999). The test statistic (T) used for this was:

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

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

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

Original score	0	1	2	3	4	5	6	7	8	9	10
New score	5	4	3	2	1	0	1	2	3	4	5

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

2.5.4.3. Modifications

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

2.5.4.4. Ethical considerations

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

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

3. Results (1)

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

3.1.1. Control diet versus CAP diet (CVC)

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

3.1.2. Control diet versus Fibre diet (CVF)

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

FIGURE TWO SHOULD GO HERE

3.2. Food versus no food discrimination trials

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

Overall, analysed at the group-level, birds in both groups showed a preference for the colour associated with food in the food versus no food trials (CVC treatment group: phase 1: $\chi^2 = 21.19$, d.f. = 1, $p < 0.001$; phase 2: $\chi^2 = 43.54$, d.f.1, $p < 0.001$; CVF treatment group: 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 significant effect of day ($F_{(6,823.0)} = 15.89$, $P < 0.001$) with birds picking the food option significantly more often than the no food option during the last few days of phases 1 and 2 indicating that they had learnt to associate the colour with food (Figures 3 and 4). There was also an effect of phase ($W_{(1,7.27)} = 7.27$ $P = 0.007$) with birds showing a stronger preference for the food over the no food option in phase 2, indicating that they found the task easier to learn the second time. There were no other significant effects or interactions (including diet option offered). In phase 1, 10/12 birds (5/6 in each treatment group individually performed better than chance in the last 20 trials (≥ 15/20 choices for the food option $p \leq 0.041$). The remaining birds selected the correct option 14/20 times. In phase 2, all birds met this criterion over the last four days.

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

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

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

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

FIGURE THREE SHOULD GO ABOUT HERE

3.3. Experimental diet versus control diet (phase 5)

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

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

4. Discussion (1)

4.1. Initial dietary preferences

The results indicated that CVC birds showed a small preference for the control diet and CVF birds did not show a preference. This suggested the CAP diet was initially less liked than the FIBRE diet or the control diet. One possibility for the failure to show any or strong preferences is that the birds consumed almost all the entire total ration (control diet plus experimental diet) within 10 minutes and thus any preference was hidden. However, an analysis of the data (not reported here) in which any bird that consumed more than 75% of the total ration (CVC birds) or 60% of the ration (CVF birds) was excluded from the analysis for the feeding session in which the cut-off point was exceeded obtained the same direction of preference (CVC group) or lack of preference (CVF birds) reported here with minimal, non-significant effects on strength or direction of any preference). Different 'cut off' points were selected for each treatment group in this alternative analysis due to the quantity of experimental diet being different between the two groups. However, the small quantities of food offered remained a serious limitation that potentially affected interpretation of the findings as the strength of any potential preference was artificially truncated. It was not possible to offer true *ad libitum* conditions due to this being a preparatory phase for the main experiment. With hindsight, one daily feeding session would have benefited data collection in view of the rapid feed consumption.

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

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

4.2. Performance during choice test

Birds found it easy to learn a food/no food discrimination task and they were able to retain this information. However they then failed to show a diet preference in the control diet versus experimental diet choice test. Side preferences more clearly explained bird performance than diet preference. It was unclear whether the development of side biases observed was a consequence of dietary option indifference, failure to associate diet type with colour, failure to transfer knowledge in the previous phases to the new, novel colour pairings or inability to distinguish between diets. To investigate these potential explanations the experimental design was modified.

5. Experimental modifications

5.1. Novel colour-pairing

5.1.1. Hypothesis

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

5.1.2. Method

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

5.2. Experimental diet versus control diet (2)

5.2.1. Hypothesis

It was assumed that if birds primarily attended to the ‘no food’ colours (i.e. they avoided the ‘no food’ option rather than specifically attended to “X” colour is associated with “X” diet option and “Y” colour is associated with “Y” diet option) then removal of this option would force attendance to the ‘food’ colours and result in discrimination between the two diet options (i.e. control diet and experimental diet). It was hypothesised that, in the continued absence of a ‘no food’ option birds would learn to associate colours with diet quality and would show a preference for the more favourable option.

5.2.2. Methods

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

5.3. Experimental diet versus control diet + 50%

5.3.1. Hypothesis

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

5.3.2. Methods

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

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

5.4.1. Hypothesis

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

5.4.2. Methods

Birds were tested on the final study day then humanely euthanized. Experimental apparatus was set up as in section 2.4.1. Each bowl was filled approximately ¾ full with either experimental or control diet which had been weighed. Within group, diet presentation was balanced for side (control diet initially on left side for 50% of birds). At 0 min birds were placed into individual pens and allowed to freely consume from both bowls. At 10 min food was removed, weighed, replenished and returned to the pens (switched to the opposite side). At 20 min the procedure was repeated. At 30 min the food was removed, weighed and discarded.

6. Results (2)

6.1. Novel colour pairing

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

FIGURE FOUR GOES ABOUT HERE

6.2. Experimental diet versus control diet (2)

6.2.1. Food preferences

As a group, birds did not exhibit a preference for either diet, either across all trials (CVC: $\chi^2 = 0.35$, $P > 0.1$; CVF: $\chi^2 = 0.23$, $P > 0.1$) or across days (Figure 5).There was no effect of treatment or day and no interaction between day and treatment. However, there was a highly significant effect of bird on the choices made ($W_{(11,3.31)} = 36.41$, $P < 0.001$). Post-hoc testing indicated that two CVC birds and two CVF birds showed a significant preference for the control diet over the 35 trials. Four CVC birds and four CVF birds failed to show a diet preference.

FIGURE FIVE GOES ABOUT HERE

6.2.2. Side biases

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

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

6.3. Experimental diet versus control diet + 50%

6.3.1. Food preferences

Although neither treatment group showed an overall preference for either the experimental 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, $P > 0.1$), there were several days on which the CVC group selected the control diet significantly more often (see figure six). However, the 'performance' of both groups was sufficiently similar that significant differences between groups were not found either overall or by day, and there was no interaction between treatment group and day.

FIGURE SIX GOES ABOUT HERE

However, there was a highly significant effect of bird on the choices made ($W_{(11,4.50)} = 49.50$, d.f. = 1, $P < 0.001$). Post-hoc testing indicated that three CVC birds and two CVF birds showed a highly significant preference for the control diet over 55 trials. A further CVC bird had a tendency to select the control diet and one CVF bird had a tendency to pick the fibre diet. The remaining five birds failed to show a diet preference, either over all 55 trials or over the last 15 trials.

6.3.2. Side biases

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

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

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

FIGURE SEVEN SHOULD GO ABOUT HERE

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

7. General discussion

7.1. Modifications

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

7.2. Observed diet preferences

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

Where significant preferences developed under closed economy, feed restricted conditions (prior to increasing control diet quantity) as they did for two CVC and two CVF birds, these preferences were also for the high quality, nutrient dense control diet. D'Eath, *et al*., (2009) suggests animals' preference for high quality feed over low quality feed might disappear under restricted feed conditions if the low quality feed confers improved satiety. Our results did not support this. However, we cannot rule out whether this was due to the experimental diets not having increased satiating effects (therefore not addressing the point) or impulsivity influencing choice by biasing any preferences towards the most rapidly consumable diet. Abeyesinghe, *et al*. (2005) found that chickens showed self-control only between a small immediate reward and a delayed (much) larger reward. This implied a need for the experimental diet to be much more rewarding if it is to be preferred. Although there was no time delay imposed on diet access, the experimental diets would take longer to consume compared to the control due to diet bulkiness (FIBRE) or additive fineness (CAP) (intake rate not measured). Anecdotal observation (unfortunately this was not formally measured) indicated that the latency to consume either experimental ration fully rapidly decreased. However, this reduction may have been concurrent with a gradual decrease in satiating capacity due to physiological adaptation to the additional dietary components (Tolkamp *et al*., 2005) further reducing its additional 'rewarding' features over the control diet. Alternatively, it is possible that the use of a schedule in which the birds alternated between the control and experimental diets created a situation in which, even if the experimental diets had increased satiating effects, because the birds were not maintained continuously on the experimental diet, the full satiating effects of these quality-adjusted diets were not achieved. Thus, the birds tested may not have been in the same physiological and / or affective state as birds reared continuously under conditions of qualitative feed restriction and this may have impacted upon both their ability to learn the task and / or to express any preference learnt.

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

7.3. Methodological issues

A long inter-trial interval (ITI) ensured birds experienced the ‘satiating’ effects of their choice through the mechanism of post-ingestion feedback. Matthews and Temple (1979), used an operant choice test to allow dairy cows to access small quantities (time restricted access ~ 5 seconds, ITI variable interval 60 – 300 seconds) of either concentrate or hay. The authors claimed this allowed diet preference quantification without the confounding variable of post-ingestion effects. However, this can be a limitation. Post-ingestion feedback shapes longer-term diet preferences (Forbes, 1998; Kyriazakis *et al*., 1999). Thus, we wanted a longer ITI with larger portions/trial.

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

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

7.4. Hunger and stress

Hunger-stress may have decreased the birds' learning ability. Although motivation to gain feed increases with degree of feed restriction (Savory *et al*., 1993; Bokkers *et al*., 2004), hunger is also a stressor (Mendl, 1999).The Yerkes-Dodson model (Yerkes and Dodson, 1908) suggests there is a bell curve effect to arousal with an optimal level of arousal for effective learning. Although the model is simplistic (Mendl, 1999) a useful basic interpretive framework is provided by it. The birds' success at learning the food-no food tasks but failure to learn the food quantity/quality choice tasks corresponded with increasing severity of feed restriction. More complex tasks have a lower optimal arousal level (Yerkes and Dodson, 1908). Stress can reinforce inflexible, habitual learning (Mitchell *et al*., 1985a) leading to poorer performance. Therefore, poor learning may have been the combined effect of being too hungry and the dietary option contrast being too small.

7.5. Side biases and stress

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

8. Conclusions

In conclusion, the selected T-maze task was not useful in investigating the feed preferences of chronically feed restricted broiler breeders. Although where birds did learn the task they preferred a small quantity of high quality feed to a quality-adjusted diet, the small number that did so limit any firm conclusions. Nevertheless, should a larger sample size replicate this preference, this would be an interesting avenue for further investigation. However, preference exploration is based on the implicit assumption that cognitive capacity to learn and exhibit a preference is not undermined by chronic hunger stress. This suggests that the impact of chronic hunger-stress on broiler breeder learning should be studied first in further investigative research focussing on feed restricted broiler breeder dietary preferences.

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Figure 1

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

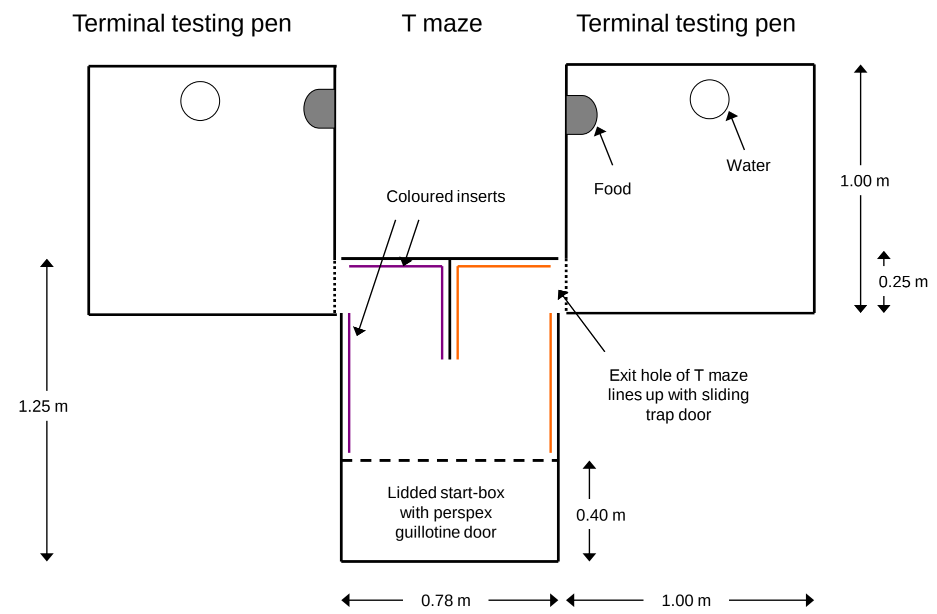

Figure 2

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).

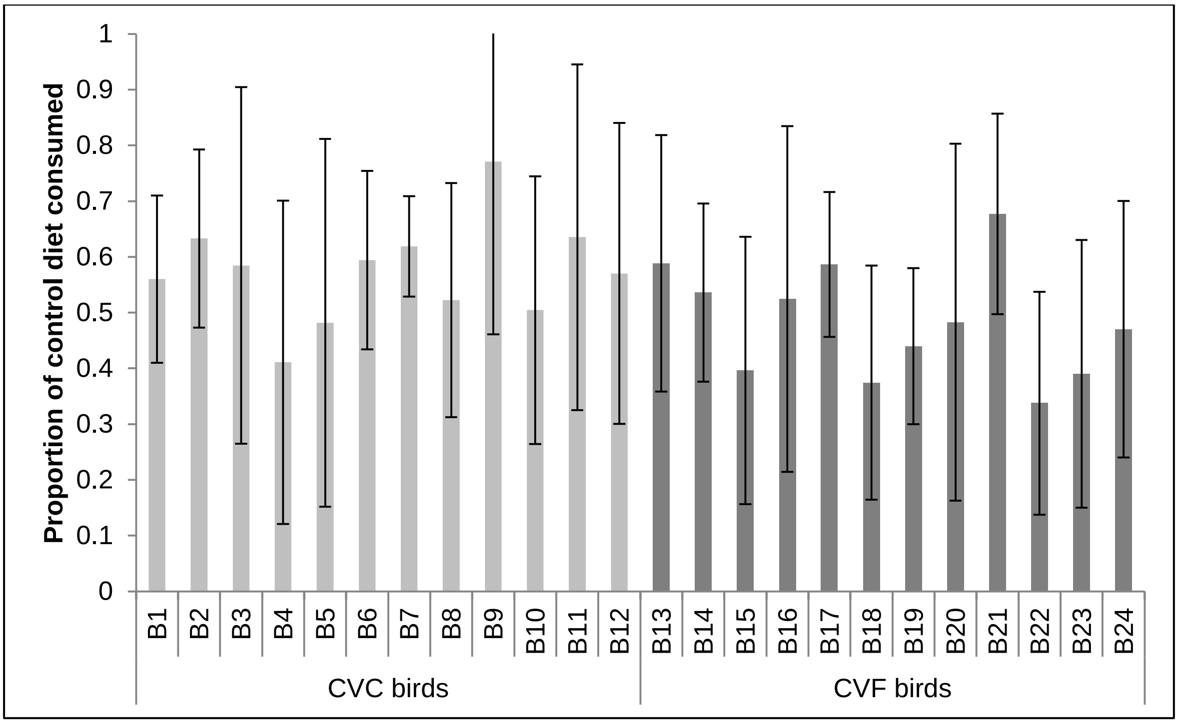

Figure 3

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 term 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 (Χ = 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.

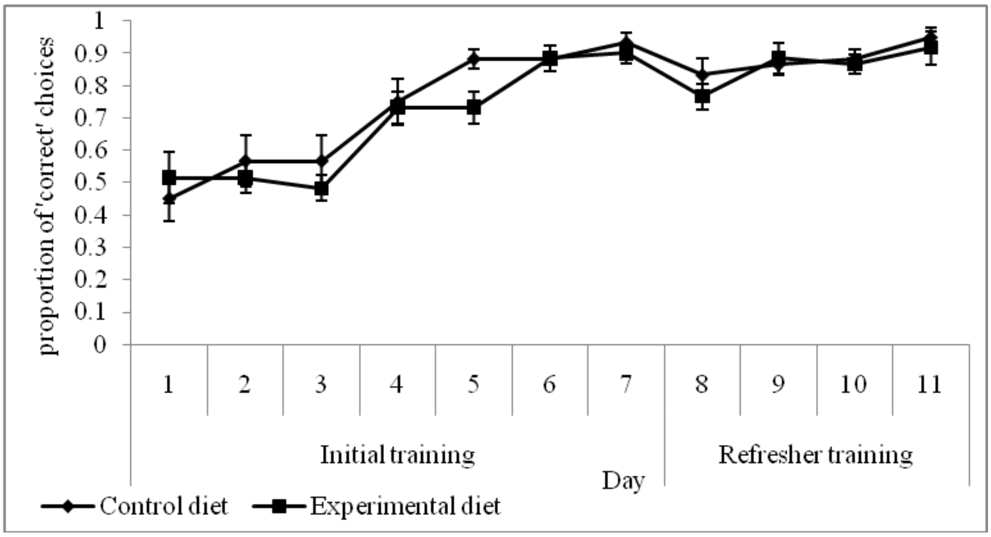

Figure 4

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.

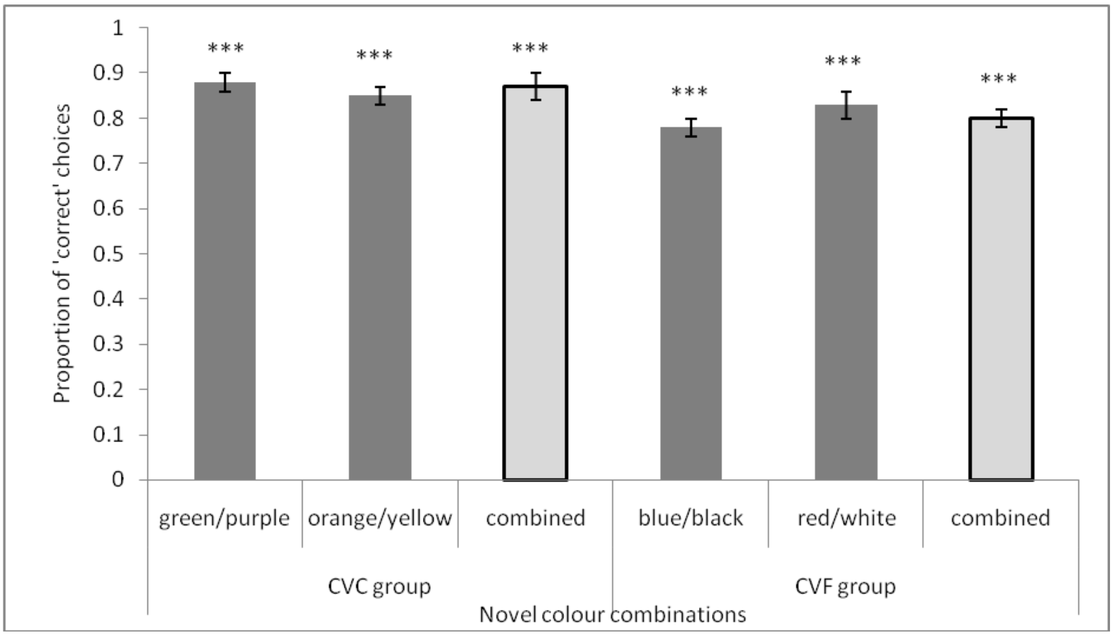

Figure 5

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.

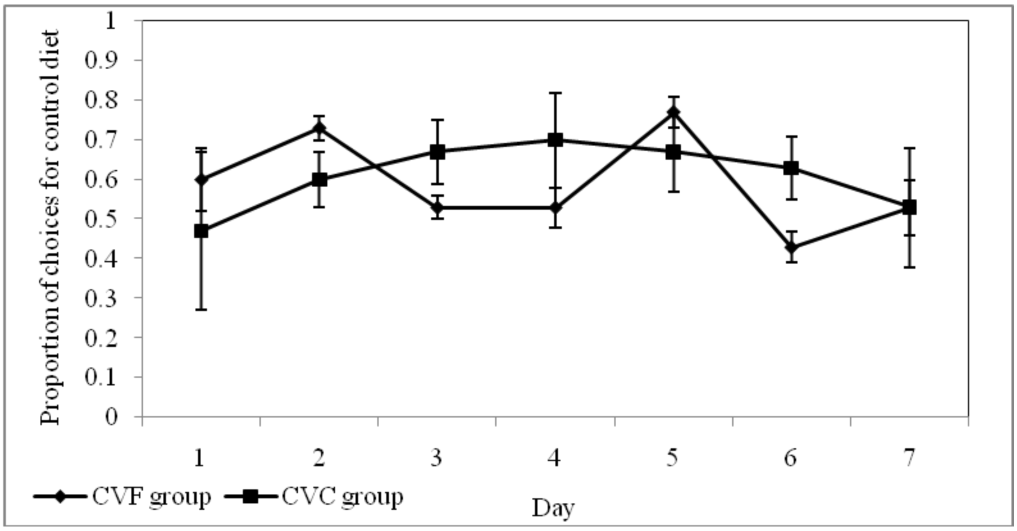

Figure 6

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 = CVC group; closed square = CVC group.

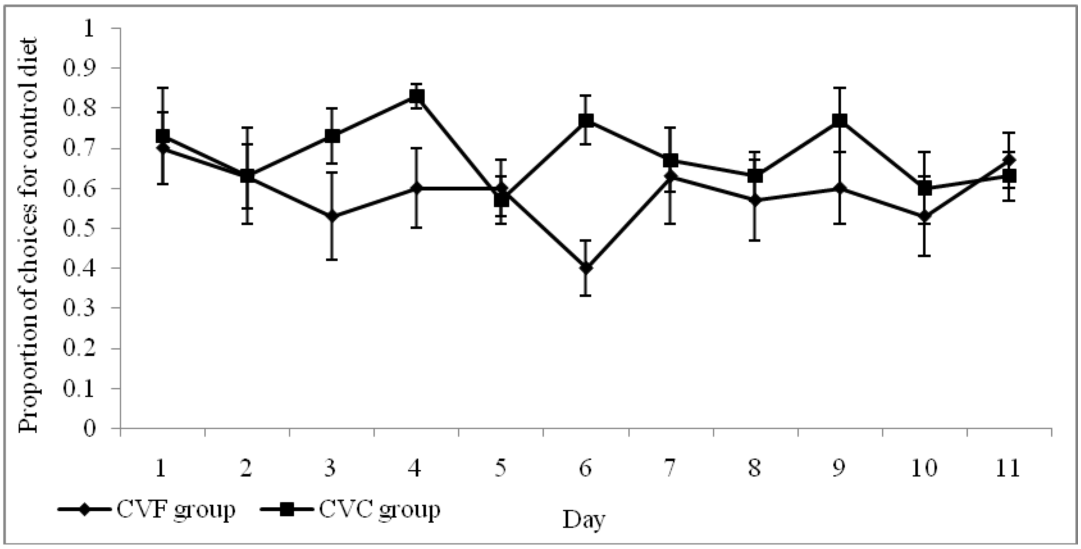

Figure 7

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..