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► **To cite this version:**

Umar Faruk Murtala, Isabelle Bouvarel, Nathalie Mème, Roffidal Lucien, Tukur Hussaini, et al.. Adaptation of wheat and protein-mineral concentrate intakes by individual hens fed ad libitum in sequential or in loose-mix systems. *British Poultry Science*, 2010, 51 (06), pp.811-820. 10.1080/00071668.2010.532772 . hal-00652139

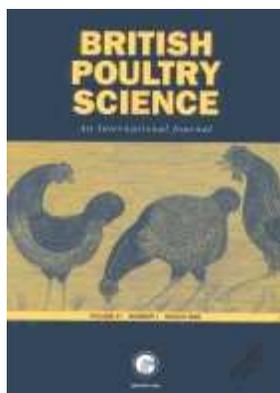
HAL Id: hal-00652139

<https://hal.science/hal-00652139>

Submitted on 15 Dec 2011

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Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2009-365.R4
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	02-Jun-2010
Complete List of Authors:	Murtala, UMAR FARUK; INRA, Tours, UR83 recherches avicoles Bouvarel, Isabelle; ITAVI Nathalie, MEME; INRA Tours, UR83 recherches avicoles Lucien, ROFFIDAL; INZO Hussaini, Tukur; Usman Danfodio University, Department of Animal Science Nys, Yves; INRA Tours, UR83 recherches avicoles Lescoat, Philippe; INRA Tours, UR83 recherches avicoles
Keywords:	sequential feeding, loose-mix feeding, Feed intake, whole-wheat, Laying hen

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Manuscripts

CBPS-2009-365

Edited Lewis September 2010, MacLeod October 2010

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2 **Adaptation of wheat and protein-mineral concentrate intakes by individual hens fed *ad libitum* in sequential or in loose-mix systems.**

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13 **Running title: Feeding system and performance in hens**
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Accepted for publication 8th June 2010

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4 41 **Abstract** 1. Feed intake and performance of birds given sequential or loose-mix feeding was
5
6 42 investigated from 19 to 42 weeks of age. A complete diet was fed as control (C). A balancer
7
8 43 diet (50) was fed either sequentially (S50) or in a loose-mix (L50) with wheat. This diet was
9
10 44 formulated to provide a similar nutritive value as C assuming a 50:50 diet and wheat intake.
11
12 45 Another balancer diet (25) was fed sequentially (S25) or in a loose-mix (L25) with wheat. The
13
14 46 diet was to provide a similar nutritive value as C assuming 75:25 diet and wheat intakes. In
15
16 47 sequential feeding, only wheat was fed in the morning (4h after lights-on) and the balancer
17
18 48 diet in the late afternoon (4h before lights-off). In the loose-mix treatment, a mixture of the
19
20 49 two diets was fed throughout the 16-h daily light. Each treatment was given *ad libitum* to 25
21
22 50 birds in individual cages.
23
24
25
26
27 51 2. Birds fed on L25 had lower total feed intakes than those receiving C, S50 or S25. Protein
28
29 52 intake was reduced with L25 compared to C, S50, S25 and L50. ME intake was, however,
30
31 53 similar among all treatments. Egg production and weight were reduced with L25 compared to
32
33 54 S50 and S25. BW was lowered with L25. However, there was high individual variation in all
34
35 55 variables.
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38
39 56 3. Feeding system (sequential vs loose-mix) had no effect on ME intake. However the loose-
40
41 57 mix treatment reduced feed and protein intake due to lower balancer diet intake. It also
42
43 58 resulted in low egg production, egg and body weights compared to sequential feeding. The
44
45 59 weights of pancreas and gizzard were heavier with sequential and loose-mix compared to the
46
47 60 control.
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51 61 4. The loose-mix treatment reduced egg-laying performance. Sequential feeding resulted in
52
53 62 similar egg-laying performance to conventional feeding and thus could be used to advantage
54
55 63 in situations where it is applicable.
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INTRODUCTION

64
65 The use of whole cereal grain in poultry feeding is a popular practice in Northern Europe. The
66 reason has been the economic benefit as well as the local availability of these feedstuffs. In
67 countries where the cost of feed mixing hinders production, direct incorporation of cereal
68 grains could be an alternative. Although the benefits depend on the relative price of cereals, it
69 is important to have a clear knowledge of the impact of incorporating cereal grains in poultry
70 feeds on bird performance. Different methods such as choice feeding, loose-mix feeding and
71 sequential feeding can be used to offer cereal grains to poultry (Noirot *et al.*, 1998). Choice
72 feeding is the simultaneous feeding of grains and a protein-mineral concentrate placed in
73 different containers. Loose-mix feeding is the distribution of these dietary components in a
74 single container. Sequential feeding involves the distribution of the two dietary components
75 separately at different times of the day. Choice feeding using cereal grains is accompanied by
76 an improvement in feed utilisation because it allows a degree of feed selection by the animal
77 (Forbes and Covasa, 1995; Noirot *et al.*, 1998). It has, however, the inconvenience of
78 requiring more than one feed trough to offer the different diets. Loose-mix and sequential
79 feeding, however, could be practical alternatives since only one trough is required.

80 Studies evaluating sequential and loose-mix feeding in laying hen are limited. Loose-
81 mix feeding of laying hens was reported to reduce both feed and protein intakes when a
82 mixture of whole wheat, whole barley, kibbled maize and pellet protein concentrate was
83 offered (Blair *et al.*, 1973), or when fed on a (50:50) mixture of high energy/protein Ca diets
84 (Lee and Ohh, 2002). Although the former observed similar egg production and egg weight to
85 conventional feeding, the latter reported a decrease in egg weight, which was related to a
86 decrease in energy intake. Loose-mix feeding of 60% barley and a protein concentrate was
87 reported to increase feed, energy and protein intakes, but to reduce egg production while
88 increasing egg weight (Bennet and Classen, 2003). However, when the quantity of whole

1
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3 89 wheat in a loose-mix diet was limited to 20%, similar intakes and egg production were
4
5
6 90 observed (Kermanshashi and Classen, 2001; MacIsaac and Anderson, 2007).
7

8 91 Sequential feeding of a mixture of whole cereals followed by a pelleted protein
9
10 92 concentrate resulted in increased feed and protein intakes while maintaining similar energy
11
12 93 intake, egg production and egg weight compared to a control diet (Blair *et al.*, 1973).
13
14 94 Nevertheless, sequential feeding was reported to reduce feed, energy and protein intakes when
15
16 95 birds were fed on a protein concentrate in the morning followed by whole oats in the
17
18 96 afternoon (Robinson, 1985), or when fed on high-energy diet in the morning and protein
19
20 97 concentrate in the afternoon (Leeson and Summers, 1978; Reichmann and Connor, 1979; Lee
21
22 98 and Ohh, 2002). Except for the work of Blair *et al.* (1973), all the other studies have reported
23
24 99 that sequential feeding results in lower egg production and egg weights.
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29 100 Recent investigations on the sequential and loose-mix feeding of whole wheat and a
30
31 101 protein-mineral concentrate in laying hen revealed that sequential feeding was more efficient
32
33 102 compared to loose-mix and the conventional feeding of a complete diet (Umar Faruk *et al.*,
34
35 103 2010). However, it was possible that this increased efficiency was linked to the experimental
36
37 104 protocol used. Feed intake was controlled by feeding a limited quantity (121 g/bird/d) of a
38
39 105 diet that contained 60.5 g each of whole wheat and concentrate. Birds were kept in groups and
40
41 106 this can facilitate social interactions among individuals and modify the birds' feeding patterns
42
43 107 through imitation (Meunier-Salaün and Faure, 1984). This management system also prevents
44
45 108 the study of individual responses to sequential and loose-mix feeding, since only the average
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47 109 cage values could be measured.
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53 110 The objective of the present experiment was to investigate the impact of sequential and
54
55 111 loose-mix feeding of birds kept in individual cages and given the diets *ad libitum*.
56
57 112 Furthermore, the study investigated the ability of birds under these feeding systems to adapt
58
59 113 their feed intake according to their requirements.
60

114 MATERIALS AND METHODS

115 **Pre-experimental period (weeks 16 – 18)**

116 A total of 149 ISA Brown layer hens were trained (Forbes and Covasa, 1995) from week 16
117 to 18 to get them habituated to the feeding systems studied. The specific objective was to
118 adapt the sequentially-fed birds to whole-wheat intakes before they reached point of lay
119 (Umar Faruk *et al.*, 2008). The animals were housed in a three-tier battery having individual
120 cages (25 x 38cm). Each cage was equipped with a feed trough (20 cm per hen) and a nipple
121 drinker. A complete diet, named 'control habituation' (Table 1) and containing 160 g CP /kg
122 and 11.7 MJ/kg, was fed to a group of 33 birds as control (CH). Another diet, named 'balancer
123 diet habituation' and containing 180 g CP /kg and 11.0 MJ/kg, was fed sequentially (SH) with
124 wheat (13.0 MJ/kg) to another group containing 58 birds. Hens in this group were given
125 access to whole wheat (*Triticum aestivum*) in the morning and to the balancer diet in the
126 afternoon. The same diet, 'balancer diet habituation', was mixed with whole wheat and fed in a
127 loose-mix (LH) to another group containing 58 birds. The 'balancer diet habituation' was
128 formulated on the assumption that if the birds consumed 65% of it and 35% whole wheat,
129 they would ingest an equal amount of nutrients as those receiving diet CH.

Table 1 near here

130 Birds were fed *ad libitum* in line with the breeders' guidelines (ISA Hendrix Genetics,
131 2007). The total quantity of diet offered was progressively increased to account for the
132 increase in feed intake of a growing bird; it was increased from 70 g/bird/d in week 16 to 83
133 g/bird/d in week 18. In sequential feeding, the duration for which birds were given access to
134 wheat was increased in equal amounts from 3 h/d (week 16) to 7 h/d (week 18). The
135 photoperiod was 10 h at week 16 and reached 16 h at week 18. Water was given *ad libitum*
136 throughout the study.

137 **Experimental period (weeks 19 - 42)**

1
2
3 138 Five treatments were introduced at 19 weeks of age. Each of the sequential and loose-mix
4
5 139 groups was divided into two groups to form 4 treatments of 25 birds each. The other 25 birds
6
7
8 140 from the CH group formed the control treatment. During the experimental period, the birds
9
10 141 were housed in the same house and kept in the individual cages that were used during the
11
12 142 habituation period. Body weight (BW) was used as criterion for the placement of the birds so
13
14
15 143 as to obtain a similar intra treatment BW. However, due to limited number of birds habituated
16
17 144 and the random choice of birds dissected for the measurement of the digestive organs, initial
18
19 145 BW for the treatment L50 was lower than C, S50 or S25. Photoperiod was 16 h and
20
21 146 temperature was maintained at $20.6 \pm 1.8^{\circ}\text{C}$ throughout the experimental period.
22
23

24 147 A complete diet (Table1) containing 175 g CP /kg and 11.5 MJ/kg was fed *ad libitum*
25
26 148 as the control treatment (C). To investigate the ability of laying hens to adapt their intake
27
28 149 according to their energy and protein requirements, the balancer diet B50 (232 g CP /kg, 10.0
29
30 150 MJ/kg) was formulated to provide a similar nutritive value to C and assuming that the birds
31
32 151 would ingest equal quantities (50:50) of the diet and whole wheat. This diet was fed to two
33
34 152 experimental groups either sequentially (S50) or as a loose-mix (L50). Another balancer diet
35
36 153 B25 (195 g CP /kg, 11.0 MJ/kg) was formulated to provide similar nutritive values as C and
37
38 154 assuming that the birds would ingest it on a 75:25 basis with wheat. It was fed to the other
39
40 155 two groups either sequentially (S25) or in loose-mix (L25). Diets were fed *ad libitum*
41
42 156 containing equal amount of each of the fractions (wheat/protein mineral concentrate). Birds
43
44 157 fed on the B50 diet were expected to have a 50% wheat intake, while only 25% wheat intake
45
46 158 was expected for those fed on the B25 diet. Calcium carbonate (Ca) was ground and
47
48 159 incorporated in the balancer diet since the separate provision of granular Ca is not a
49
50 160 prerequisite when birds are fed on nutrient-fractioned diets (Balnave and Muritasari, 1990).
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57 161 **Measurements**

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3 162 Feed intake was measured weekly. In the two S fed treatments, wheat and balancer diet
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5 163 intakes were determined separately. During the experimental period, wheat intake in L was
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8 164 measured after manual separation using a sieve (2 mm Φ).
9

10 165 Eggs were collected daily and weighed individually. The weight of egg components
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12 166 (yolk, albumen and shell) was determined on a daily sample every 4 weeks, starting at week
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14
15 167 21. The albumen and the chalazae were separated using forceps prior to weighing the yolk.
16
17 168 The shells were washed and dried for 12 h in a drying oven at 70°C and then weighed.

18
19
20 169 Birds were weighed individually at 16, 19, 26 and 37 weeks of age. The weight of the
21
22 170 digestive organs was recorded at the end of the pre-experimental (week 19) and experimental
23
24 171 (week 42) periods to assess the effect of the feeding system on these organs. The birds were
25
26 172 weighed before being injected with sodium pentobarbital solution (1ml/kg body weight). The
27
28 173 abdominal cavity was opened and the digestive tract dissected and separated into
29
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31 174 proventriculus, gizzard, duodenum, pancreas, jejunum and ileum. The segments were first
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33 175 emptied and dried using a paper towel before weighing. The proventriculus and the gizzard
34
35 176 were placed in an ice container (-4°C) for 3 h to facilitate the removal of the surrounding fat.
36
37
38 177 The organs were emptied prior to weighing.
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41 178 Metabolisable energy (kJ/b/d) and protein (g/b/d) intakes were estimated as a product
42
43 179 of feed intake and ME and protein contents of the experimental diets respectively. ME
44
45 180 requirement was estimated according to Sakomura (2004)
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47

48 181
$$ME \text{ (kJ/b/d)} = W^{0.75} * (165.74 - 2.37 * T) + 6.68 * WG + 2.40 * EM$$

49
50 182 where, *ME* = energy requirement (kJ/bird/d), *W* = Body weight (kg), *T* = Temperature (°C),
51
52 183 *WG* = weight gain (g/d), *EM* = Egg mass (g/bird/d).
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54

55 184 Protein requirement was estimated according to Sakomura *et al.*, (2002)

56
57 185
$$PB = 1.94 * W^{0.75} + 0.480 * G + 0.301 * EM$$

1
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3 186 where, PB = protein requirement (g/bird/d), W = body weight (kg), G = daily weight gain
4
5 187 (g/d) and EM = egg mass (g/bird/d).
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8 188 **Statistical analysis**

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10 189 Individual values from cages were analysed using StatView (version 5, SAS Institute Inc.,
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12 190 Cary, NC). A one-way ANOVA was performed using the GLM model to test the treatment
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14 191 effect on feed intake, egg production, egg weight, body weight, digestive organs weight, ME
15
16 192 intake and requirements. A 2x2 factorial ANOVA was carried out to test the effect of feeding
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18 193 system (loose-mix *vs* sequential) and balancer diet effect (50 *vs* 25). Results were considered
19
20 194 significantly different if $P < 0.05$ and Bonferroni-Dunnett pairwise comparison was used to
21
22 195 compare differences between means. Repeated measures ANOVA was performed on BW,
23
24 196 feed and wheat intakes, and egg mass to test the effect of treatment over time.
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27
28

29 197 **RESULTS**

30
31 198 During the habituation period, no differences in total feed intakes were observed between the
32
33 199 treatments (62.4, 62.5 and 62.4 ± 0.3 g/bird/d for CH, LH and SH respectively). The wheat
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35 200 intakes of the birds given the SH treatment increased from 13.0 to 40.0 g/bird/d from week 16
36
37 201 to 18 respectively. Their balancer-diet intake, however, decreased with increasing age and
38
39 202 wheat intake from 51.6 g/bird/d in week 16 to 40.0 g/bird/d in week 18. This increase in
40
41 203 wheat intake with the resulting decrease in balancer diet intake during this period suggested a
42
43 204 successful adaptation to wheat intake for SH-fed birds. There were no differences in BWG
44
45 205 between week 16 and week 18 (8.1, 8.3, and 8.6 ± 0.1 g/bird/d for CH, LH and SH
46
47 206 respectively).
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52
53 207 During the experimental period, the mean total feed intake was lower for birds given
54
55 208 the treatment L25 than C, S50 and S25, although it was similar to L50 (Table 2). Figure (b)
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57 209 shows the weekly change in feed intake according to treatment from 19 to 42 weeks. There
58
59 210 was a significant increase in feed intake in all treatments with time, although birds fed on L25
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3 211 and L50 had lower feed intakes from week 30 of age but with a higher weekly variability than
4
5 212 the other three treatments. Wheat intake was higher for birds given L50, L25, S50 and S25
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7
8 213 (Table 2). There was a progressive but significant decrease in wheat intake over time,
9
10 214 especially with treatments S25 and S50 (Figure c).

11
12 215 Egg production and egg weight were reduced with L25 compared with S50 and S25
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14
15 216 (Table 2). Egg production and weight were, however, not statistically different between L25,
16
17 217 L50 and C. Birds in treatment L25 had a significantly smaller egg mass and lower egg yolk
18
19 218 weight compared with the other 4 treatments. Egg mass in this treatment was progressively
20
21 219 reduced from week 23 and was generally low through out the experimental period (Figure d).
22
23 220 Eggshell weight was significantly reduced with L25 when compared with S25, S50 and L50.
24
25 221 There were no significant treatment differences for albumen weight. Feed conversion ratios
26
27 222 (FCR) were similar among all treatments.

Table 2 and Figure near here

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30
31 223 BW at week 19 was significantly lower for birds receiving L50 than for C, S50 and
32
33 224 S25, although they were similar to L25 (Table 2); this was associated with the low BW of the
34
35 225 treatment group upon arrival at week 16 (Figure a). Although L50 was initially low in BW,
36
37 226 the final BW (week 42) was lower for L25 compared with C, S25 and S50. The Figure (a)
38
39 227 shows that the birds which received L50 had a similar growth rate to the other treatments.
40
41 228 Conversely, L25, which was similar in BW to all treatments at the beginning of the
42
43 229 experiment, had reduced growth, thus having a lower BW than C, S50 and S25 at the end of
44
45 230 the experiment.

46
47 231 Feeding system affected the birds' feed intakes and egg-laying performance and the
48
49 232 birds fed sequentially had higher total feed intakes than those fed on the loose-mix (Table 2).
50
51 233 The latter had higher wheat intakes. Hens fed sequentially had higher egg numbers, egg
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53 234 weight and albumen weight than those fed on the loose-mix. BW was higher in the birds fed
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3 235 sequentially than with those fed on loose-mix and this was associated with the higher initial
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5 236 BW. FCR and BWG were similar for the two systems.

7 237 Birds receiving balancer diet optimised for 25% wheat intake had lower mean total
9
10 238 feed and wheat intakes compared with those receiving diet optimised for 50% (Table 2). Egg
11
12 239 numbers, egg weight, egg albumen weight, FCR and final BW were similar for these two
14
15 240 diets. However, BWG was higher in the birds fed on balancer diet optimised for 50% wheat
16
17 241 intake compared with that optimised for 25%.

19 242 There was a similar ME intake for all treatment groups (Table 3). Estimated ME
20
21 243 requirement for treatment L25 was lower than that of C, S50 and S25 but similar to L50. The
22
23 244 difference between ME intake and ME requirement was higher for birds fed on L25 than S50,
24
25 245 S25 or C, although it was similar to L50, indicating a surplus in ME intake of birds in these
26
27 246 treatment groups. Protein intake was reduced with L25-fed birds compared with the other 4
28
29 247 treatments. Protein intake of birds fed on L50 was lower than C and S50 but similar to S25.
30
31 248 The estimated protein requirement was reduced in birds fed on L25 compared with C, S50
32
33 249 and S25 but similar to L50. The difference between protein intake and requirement was small
34
35 250 for birds fed on L25 and S25 compared with those fed on S50. Table 3 near here

37
38
39 251 ME intake was similar for the sequential and loose-mix systems (Table 3). Estimated
40
41 252 ME requirement of sequentially fed birds was significantly higher than the loose-mix group.
42
43 253 The difference between ME intake and estimated requirement was lower for birds fed
44
45 254 sequentially. Both intake and estimated requirement of protein were significantly higher in
46
47 255 birds fed sequentially than in those fed on loose-mix.

48
49
50 256 ME intake and estimated requirements and the differences between ME intake and
51
52 257 estimated requirement were similar between the two balancer diets (Table 3). Protein intake
53
54 258 was low for birds receiving the balancer diet formulated for 25% wheat intake relative to
55
56
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259 those receiving the diet with balancer formulated for 50%. Protein requirement was similar
260 for the two diets.

261 At the end of habituation period (week 19), similar relative weights of proventriculus,
262 liver and pancreas were observed between CH, SH and LH (Table 4). The relative weight of
263 the duodenum was lower for birds in the LH group than SH. The relative weight of the ileum
264 was heavier for birds in SH group than with those in LH and CH. There were significant
265 differences in gizzard and jejunum weights between the treatment groups, but the differences
266 were not proven using the Bonferroni-Dunnet pairwise comparison test due, probably because
267 of the limited number of birds killed (8/treatment).

Table 4 near here

268 At the end of the experimental period (week 42), the proventriculus was heavier in the
269 birds given S than the C and L treatments; the gizzard was heavier for the S and L treatments
270 compared with C; the duodenum was heavier for S compared with C; the liver was heavier
271 with the L treatment compared with C and the pancreas was heavier for S and L treatments
272 compared with C. There were no significant differences in the weights of the jejunum and
273 ileum between the three treatments.

274 DISCUSSION

275 None of the 4 treatments receiving whole wheat had a daily wheat intake that met the
276 expected 25 or 50% of the total daily feed intake. The birds receiving the diet formulated for
277 50% wheat intake consumed slightly less wheat (47%) than anticipated, whereas those fed on
278 the diet formulated for 25% wheat intake consumed more wheat (41%). However, no
279 treatment differences in ME intake were observed, which suggests that there was an
280 adjustment by the sequential and loose-mix fed birds to consume a similar ME to those fed on
281 the complete diet C. This lends support to work of Blair *et al.* (1973), Kermanshashi and
282 Classen (2001) and MacIsaac and Anderson (2007), who reported similar ME intakes in birds
283 fed on a loose-mix or complete diet. However, in sequential feeding, Leeson and Summers

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2
3 284 (1978), Reichmann and Connor (1979), Robinson (1985), and Lee and Ohh (2002) reported a
4
5 285 decrease in ME intake, along with a reduced feed intake, which was not the case in the present
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7
8 286 work.

9
10 287 Birds fed sequentially had higher feed intakes compared with the loose-mix treatment,
11
12 288 but this was not in accordance to our earlier work with birds housed in groups (Umar Faruk *et*
13
14 289 *al.*, 2010), where a higher feed intake was obtained with loose-mix than with sequential
15
16
17 290 feeding. In this earlier work, the birds were housed in groups and fed on limited quantities of
18
19
20 291 the two dietary fractions. The lower feed intakes with loose-mix in the present experiment
21
22 292 was mainly attributed to treatment L25, because of its similar balancer diet intake compared
23
24 293 to L50. Although L25 fed birds consumed more wheat than expected, their balancer diet
25
26
27 294 intake remained similar to L50, thereby reducing their total intake.

28
29 295 The higher wheat intake when birds were fed with a loose-mix relative to those fed
30
31 296 sequentially agree with earlier observations under different conditions (Umar Faruk *et al.*,
32
33 297 2010). Increased wheat intake has been associated with larger feed particle preference in
34
35
36 298 laying hens (Picard *et al.*, 1997; Umar Faruk *et al.*, 2008). Portella *et al.* (1988) observed a
37
38
39 299 greater disappearance of larger particles when birds were fed on regular crumbles, and the
40
41 300 smaller particles disappeared only as the concentration of large ones decreased. In the present
42
43
44 301 work, the inclusion of whole wheat in the concentrate diet is likely to have increased the
45
46 302 heterogeneity of the diet, thus increasing selection of the larger particles (whole wheat) first.

47
48 303 Birds fed sequentially consumed more balancer diet than those fed on a loose-mix.
49
50 304 Blair *et al.* (1973) observed an increased feed intake when birds were fed sequentially and, in
51
52
53 305 particular, when there was a high intake of protein concentrate. However, sequential feeding
54
55 306 using an energy-rich diet in the morning and a protein concentrate in the afternoon (Leeson
56
57
58 307 and Summers, 1978; Reichmann and Connor, 1979) reduced feed intake. Feed intake was also
59
60 308 reduced when oats/sorghum were sequentially fed with a protein concentrate (Robinson,

1
2
3 309 1985). Lee and Ohh (2002) also reported a reduction in feed intake when birds were fed on a
4
5 310 high energy/protein and low Ca diet in the morning followed by a low energy/protein and
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7 311 high Ca in the afternoon. In the present work, the high balancer diet intake with the sequential
8
9 312 feeding agreed with the feeding patterns observed with laying hens (Ballard and Biellier,
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11 313 1969; Nys *et al.*, 1976; Keshavarz, 1998*a,b*; Choi *et al.*, 2004). Birds consume larger amount
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13 314 of food in the afternoon partly in association with the hen Ca appetite which coincides with
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15 315 the initiation of eggshell formation during this period. For example, Keshavarz (1998*a*) found
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17 316 that hens subjected to a 16-h photoperiod consumed 40% of daily feed intake in the first 8 h
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19 317 after light-on and 60% during the second 8 h period before light-off, with a marked increase
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21 318 recorded 4 h before light-off (Keshavarz, 1998*b*).

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27 319 The laying hen adapts its feed intake relatively well to the energy value of its feed,
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29 320 although this regulation is not perfect (Joly and Bougon, 1997), because the hen is influenced
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31 321 by the form and method in which the feed is presented. Data in the present work were
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33 322 subjected to a *t*-test to see if the ME intake minus ME requirement were different from zero.
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35 323 Results showed that no difference between the ME intake and the ME requirements for the
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37 324 birds in groups C as well as L50, suggesting that the two groups have adapted their ME intake
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39 325 to their estimated requirements. However, birds fed on S50 and S25 had lower ME intakes
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41 326 than the estimated requirements. L25 had a significantly higher intake than requirement,
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43 327 although their efficiency of utilising this energy was apparently poor because they had the
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45 328 lowest performance. Despite their lower-than-estimated ME intake, the birds fed on S50 and
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47 329 S25 had similar egg-laying performance to those fed on the control diet. This was also
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49 330 observed in previous work (Umar Faruk *et al.*, 2010) and in the earlier work of Leeson and
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51 331 Summers (1978) and Reichmann and Connor (1979), who observed that sequential feeding
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53 332 reduced the mean feed intake without reducing egg production, relative to the control. These
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55 333 authors associated it to lower maintenance requirements due to the lower body weights in the
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3 334 sequentially fed birds. In the present work, it may be due to an improvement in the rate of
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5 335 utilisation of nutrients evidenced by a heavier gizzard, proventriculus and duodenum observed
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7
8 336 with sequential than the loose-mix or control birds.
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10 337 Loose-mix-fed birds laid fewer eggs and had a lower egg weight than those fed
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12 338 sequentially. A reduction in protein intake is usually accompanied by a reduction in both egg
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15 339 production and egg weight (Morris and Gous, 1988), and protein intakes were lower with
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17 340 loose-mix treatment. *Ad libitum* access to the diets gave higher wheat consumption in the
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20 341 loose-mix birds (Umar Faruk *et al.*, 2008), which resulted in a low overall protein intake.
21

22 342 At the end of the experimental period, the sequential and loose-mix fed birds had
23
24 343 heavier gizzards than those receiving the control diet. This was to be expected because
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27 344 feeding whole wheat grain increases the dietary particle size of the diets, and dietary particle
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29 345 size is known to influence the avian digestive tract (Nir *et al.*, 1990). This increase in gizzard
30
31 346 weight was hypothesised to contribute to the better performance of sequentially-fed, group-
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34 347 housed hens studied by Umar Faruk *et al.* (2010). In the present work, no improvement in
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36 348 performance was observed; this was likely to be a consequence of the broad intra-treatment
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38 349 variations and the *ad libitum* feeding. Changes in liver weight were in line with the findings of
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41 350 De Basilio *et al.* (2001), who found heavier livers in broilers that had been fed on cereal
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43 351 grains. It was also in line with the observations of Umar Faruk *et al.* (2010), who observed
44
45
46 352 heavier liver weights in birds fed on whole wheat.
47

48 353 In conclusion, the present study confirmed our earlier report (Umar Faruk *et al.*, 2010)
49
50 354 that sequential feeding has no adverse effect on egg production, thus outlining its potential as
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53 355 a feed management method for egg producers. When birds are allowed *ad libitum* access to
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55 356 concentrate diets formulated for different proportion of wheat intake, it is evident that they
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58 357 will not always consume the expected proportion of the respective fractions. In sequential
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60 358 feeding, similar feed intakes and egg-laying performance to the control group were obtained

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3 359 irrespective of the concentrate diet used. This system could therefore be used to advantage in
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5 360 situations where the cost of grinding and feed mixing hinders production. In loose-mix
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8 361 feeding, distributing concentrated diets optimised for 25% wheat intake reduced nutrient
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10 362 intake and performance as the hens failed to eat the appropriate proportion of the dietary
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13 363 fractions, and so this management technique should be avoided.

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15 364 The advantages of sequential feeding needs to be further explored in terms of the
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17 365 physical form (whole, ground) and type of cereals (millet, sorghum, maize etc) to be used. *Ad*
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19 366 *libitum* feeding in sequential feeding resulted in excessive intake of the concentrate diet and
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21
22 367 this may increase cost and so requires additional investigation. Feeding management (van
23
24 368 Krimpen *et al.*, 2005), especially sequential feeding (Jordan *et al.*, 2009), induces feather
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27 369 pecking in laying hens, due to a reduction in time spent on feeding activity. Therefore, the
28
29 370 optimal duration of the sequence (wheat-balancer diet) in sequential feeding needs to be
30
31 371 established.

32 33 34 372 ACKNOWLEDGEMENTS

35
36 373 The authors acknowledge the technical assistance given by Jean-Marc Hallouis, Anne-Marie
37
38 374 Chagneau, Maryse Leconte and Serge Mallet. They also thank Lucille Delestre, Valentine
39
40
41 375 Froget and Amandine Soria for their help in data collection. The help of our experimental unit
42
43 376 (UE PEAT) in the set-up of this experiment was highly appreciated. Finally we thank France
44
45 377 AgriMer, CNPO and INZO° for their financial support.

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Table 1. *Composition of experimental diets*

Ingredient (g/kg)	Habituation Period (16-18 week)		Experimental Period (19-42 week)			Both periods
	Control habituation (CH)	Balancer diet habituation (LH)	Control (C)	Balancer diet		Whole Wheat (<i>Triticum aestivum</i>)
				B50	B25	
Wheat	346.6	-	500.0	-	-	100
Maize	350.0	535.7	161.3	320.8	498.4	
Wheat bran	100.0	153.1	25.4	50.1	45.0	
Maize gluten	-	-	32.9	66.2	24.0	
Soya bean meal (T48)	165.0	252.5	169.7	340.8	285.0	
Soya bean oil	-	-	8.0	16.0	10.0	
Calcium carbonate	18.4	28.2	80.0	160.4	105.1	
Bi-calcium phosphate	10.9	16.7	11.6	23.3	17.4	
Refined sodium chloride	2.0	3.1	2.0	4.0	2.7	
Sodium bicarbonate	2.0	3.1	2.0	4.0	2.7	
L-Lysine 78	-	-	1.1	2.2	1.3	
DL-Methionine	0.1	0.2	1.1	2.2	1.8	
Premix (Sup 64 J 02) ¹	5.0	7.7	5.0	10.0	6.7	
<i>Calculated composition (g/kg as fed basis)</i>						
Metabolisable energy (MJ/kg)	11.7	11.0	11.5	10.0	11.0	13.0
CP	160.5	183.3	175.2	233.2	194.7	119.0
DM	877.0	875.0	891.0	899.0	889.0	868.0
Fibre	35.9	41.0	30.1	33.7	32.7	26.5
Lysine	7.2	9.3	8.1	13.1	10.7	3.1
Methionine	3.2	3.9	4.5	7.1	5.7	2.0
Calcium	12.0	18.2	36.1	72.0	48.0	0.3
Total P	5.6	7.1	5.3	7.6	6.4	3.0
<i>Analysed composition (g/kg)</i>						
DM	890.0	887.0	888.0	895.0	890.0	867.0
CP	157.0	186.0	173.0	230.0	195.0	119.0

¹Vitamin and mineral premix supplied the following amounts per kg of premix: 1200 mg of Cu (sulphate), 4000 mg of Fe, 200 mg of I, 60 mg of Se, 120 g of DL-methionine, 200 mg of canthaxanthine; 11000 mg of Zn, 12000 mg of Mn; retinol 480 mg, cholecalciferol 12 mg, DL α -tocopherol acetate 2000 mg, menadione 400 mg, thiamine mononitrate 109 mg.

Table 2. Effect of treatment, feeding system and balancer-diet composition on the feed intake and performance of hens fed whole wheat sequentially (S) or in loose-mix (L) with a balancer diet formulated for either 50 or 25% wheat intake from 19 to 42 weeks of age

	Feed Intake (g/b/d)	Wheat intake/Feed intake	Eggs produced per hen per d	Egg weight (g)	Egg mass (g/d)	FCE (g egg/ g feed)	BW wk 19 (g)	BW wk 42 (g) ²	BWG (g/d)	Egg Yolk (g)	Egg Albumen (g)	Eggshell (g)
Treatments ¹												
C	114.4 ab	-	0.946 ab	57.3 ab	54.2 a	0.473	1560 a	1832 a	1.7 a	14.2 a	36.9	6.0 ab
S50	115.7 a	0.384 c	0.950 a	58.2 a	55.5 a	0.481	1588 a	1810 a	1.4 ab	14.0 a	38.0	6.1 a
S25	111.5 ab	0.315 d	0.947 a	58.4 a	55.3 a	0.498	1603 a	1809 a	1.1 ab	14.1 a	38.0	6.3 a
L50	107.0 bc	0.563 a	0.924 ab	57.2 ab	53.4 a	0.486	1485 b	1722 ab	1.4 ab	14.0 a	37.2	6.1 a
L25	102.8 c	0.502 b	0.886 b	55.0 b	49.0 b	0.476	1545 ab	1622 b	0.6 b	13.2 b	35.4	5.7 b
<i>P</i> value	<0.01	<0.01	<0.05	<0.05	<0.01	NS	<0.01	<0.05	<0.05	<0.05	NS	<0.05
SEM	0.97	1.20	0.69	0.35	0.50	0.004	8.10	17.73	0.09	0.09	0.30	0.04
Feeding system ²												
L	104.8 b	0.532 a	0.904 b	56.0 b	-	0.480	1515 b	1688 b	1.1	-	36.4 b	-
S	113.7 a	0.350 b	0.948 a	58.3 a	-	0.490	1596 a	1810 a	1.3	-	38.0 a	-
<i>P</i> value	<0.01	<0.01	<0.05	<0.05	-	NS	>0.01	<0.05	NS	-	<0.05	-
SEM	1.11	1.20	0.83	0.40	-	0.005	9.52	20.23	0.09	-	0.34	-
Balancer Diet effect												
50	111.3 a	0.474 a	0.937	57.8	-	0.483	1535 b	1766	1.4 a	-	37.7	-
25	107.0 b	0.412 b	0.916	56.6	-	0.487	1573 a	1716	0.9 b	-	36.9	-
<i>P</i> value	<0.05	<0.05	NS	NS	-	NS	<0.05	NS	<0.05	-	NS	-
SEM	1.11	1.20	0.83	0.40	-	0.005	9.52	20.23	0.09	-	0.34	-
Interaction												
FS x DC	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	<0.05	NS	<0.05

¹C = Control, S50 = Sequential feeding of diet formulated for 50% wheat, S25 = Sequential feeding of diet formulated for 25% wheat, L50 = Loose-mix feeding of diet formulated for 50% wheat, L25 = Loose-mix feeding of diet formulated for 25% wheat.

²Results were not presented where interaction between feeding system and balancer diet effect were observed

^{a,b,c} Values within the same column not sharing a common superscript differ significantly at $P < 0.05$, NS = not significant at $P > 0.05$.

Table 3. Effect of treatment, feeding system and balancer-diet composition on the feed, ME and protein intakes and ME and protein requirements of hens fed whole wheat sequentially (S) or in loose-mix (L) with a balancer diet formulated for either 50 or 25% intake of wheat from 19 to 37 weeks of age¹

	Wheat				Difference	Protein	Protein	Difference
	Feed intake	intake/Feed	ME intake	ME Requirement	ME	intake	Requirement	Protein
	(g/b/d)	intake	(kJ/b/d) ²	(kJ/b/d) ³	(kJ/b/d) ⁴	(g/b/d)	(g/b/d)	(g/b/d)
Treatments ⁵								
C	113.1 a	-	1300.0	1321.4 a	-21.1 b	19.8 b	19.9 a	-0.1 ab
S50	113.6 a	0.387 c	1264.2	1311.0 a	-47.0 b	21.4 a	20.1 a	1.3 a
S25	109.9 a	0.320 d	1280.0	1308.1 a	-28.2 b	18.7 bc	20.0 a	-1.3 b
L50	106.6 ab	0.569 a	1274.0	1241.0 ab	33.0 ab	18.4 c	18.5 ab	-0.1 ab
L25	101.5 b	0.503 b	1226.0	1178.1 b	47.7 a	15.9 d	17.4 b	-1.5 b
<i>P</i>	<0.01	<0.01	NS	<0.01	<0.05	<0.01	<0.01	<0.01
SEM	1.00	1.17	9.20	10.30	9.00	0.23	0.23	0.22
Feeding system ⁶								
L	103.9 b	0.533 a	1248.0	1207.0 b	41.0 a	17.1 b	18.0 b	-
S	111.8 a	0.354 b	1272.0	1310.0 a	-37.7 b	20.1 a	20.0 a	-
<i>P</i>	<0.05	<0.01	NS	<0.01	<0.01	<0.01	<0.01	-
SEM	1.12	1.17	10.50	11.61	10.60	0.27	0.27	
Balancer Diet effect								
50	110.4 a	0.472 a	1269.0	1279.2	-11.0	20.0 a	19.4	-
25	105.5 b	0.415 b	1252.3	1242.0	11.0	17.3 b	18.7	-
<i>P</i>	<0.05	<0.05	NS	NS	NS	<0.01	NS	-
SEM	1.12	1.17	10.50	11.61	10.60	0.27	0.27	
Interaction								
FS x DC	NS	NS	NS	NS	NS	NS	NS	<0.05

¹Values shown are averages from week 19 to 37 because body weight at week 42 was only measured for the birds used in the measurement of digestive organs.

²Estimation of ME (KJ/b/d) and protein (g/b/d) intakes were calculated as the product of feed intake and diet composition.

³Requirements in ME (kJ/b/d) and protein (g/b/d) were estimated according to Sakomura (2004) and Sakomura *et al.* (2002) respectively.

⁴Difference between requirement and intake were estimated as intake minus requirement.

⁵C = Control, S50 = Sequential feeding of diet formulated for 50% wheat, S25 = Sequential feeding of diet formulated for 25% wheat, L50 = Loose-mix feeding of diet formulated for 50% wheat, L25 = Loose-mix feeding of diet formulated for 25% wheat.

⁶Result were not presented where interaction between feeding system and balancer diet effect were observed.

^{a,b,c} Values within the same column not sharing a common superscript differ significantly at $P < 0.05$, NS = not significant at $P > 0.05$.

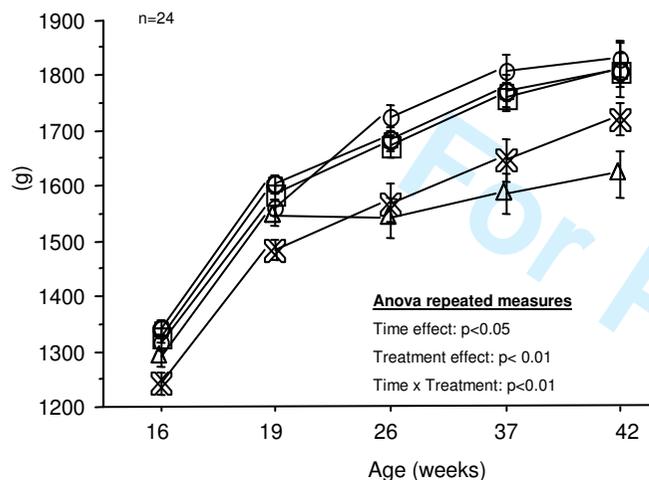
Table 4. Effect of feeding system on weight of digestive organs (g/kg body weight) at weeks 19 and 42 of birds fed whole wheat sequentially or in loose-mix with a balancer diet

Organ (g/kg)	Week 19					Week 42 ¹					
	Feeding system ²			P value	SEM	Feeding system			P value	SEM (n=16)	SEM (n=24)
	CH (n=8)	SH (n=8)	LH (n=8)			C (n=16)	S (n=24)	L (n=24)			
Proventriculus	3.1	3.1	2.8	NS	0.08	3.3 b	3.7 a	3.3 b	<0.05	0.11	0.08
Gizzard	18.5	21.9	21.7	<0.05	0.62	11.8 b	13.8 a	14.7 a	<0.05	0.48	0.37
Duodenum	5.4 ab	6.4 a	5.1 b	<0.05	0.21	5.2 b	5.9 a	5.5 ab	<0.05	0.20	0.18
Jejunum	8.5	10.1	8.5	<0.05	0.30	9.4	9.5	9.7	NS	0.26	0.24
Ileum	6.3 b	7.5 a	6.2 b	<0.05	0.22	7.6	7.2	7.4	NS	0.25	0.19
Liver	24.7	25.1	24.4	NS	0.74	25.5 b	26.8 ab	28.9 a	<0.05	0.76	0.91
Pancreas	2.1	2.2	2.2	NS	0.06	1.6 b	1.8 a	1.8 a	<0.05	0.06	0.06

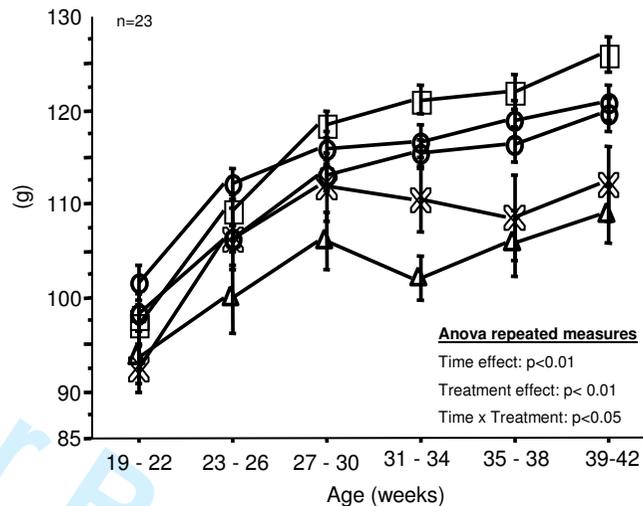
¹No difference in organs weight between treatments of the same feeding system and no interaction (feeding system x balancer diet level) was observed. Therefore, animals receiving diet 25 were put together with their corresponding 50 treatments to increase the population size from 16 to 24.

²CH= Control Habituation, SH= sequential habituation, LH= loose-mix habituation, C = Control, L = Loose-mix, S = Sequential
a,b,c: Values within the same row not sharing a common superscript differ significantly with Bonferroni-Dunnet ($P < 0.05$), NS = not significant at $P > 0.05$.

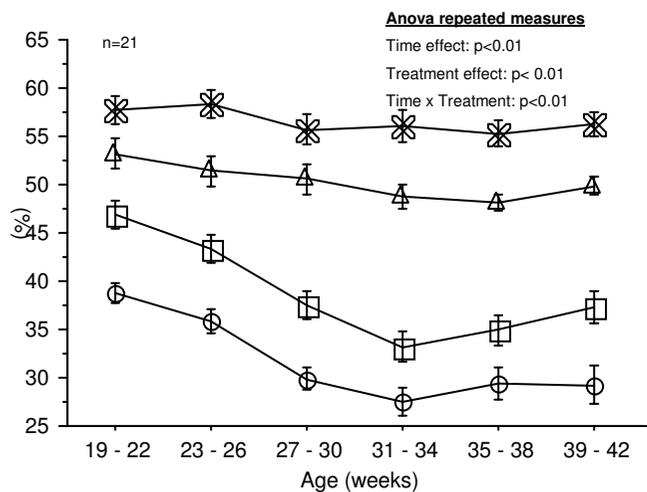
a. Body weight (g)



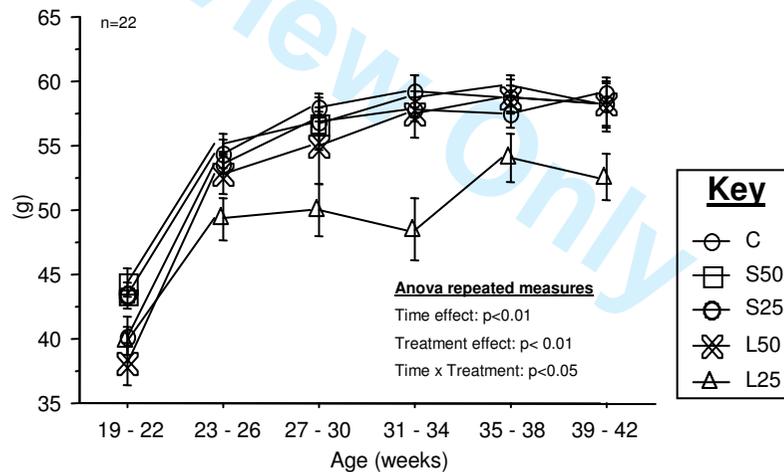
b. Feed intake (g/bird/d)



c. Wheat intake (% total feed intake)



d. Egg mass (g/d)



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Figure. *Individual variation within treatment in (a) BW, (b) Feed intake, (c) wheat intake and (d) egg mass of birds fed whole wheat sequentially (S) or in loose-mix(L) with a balancer diet formulated for either 50 or 25% intake of wheat from 19 to 42 weeks of age.*

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