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1           **Species-dependent response to the influence of adaptation length during assay for**  
2           **metabolisable energy of cereal grains employing the difference method**

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18 ABSTRACT

19 Three experiments were conducted to determine the influence of varying lengths of adaptation  
20 on metabolisable energy (AME and AMEn) content of maize and barley for broilers, turkeys and  
21 laying hens using the difference method. Three hundred and twenty-four Cobb 500 male broiler  
22 chicks (Experiment 1), 162 BUT 10 male turkey poults (Experiment 2) or 162 Lohmann brown  
23 laying hens (Experiment 3) were offered a nutrient-adequate pre-experimental diet for at least 11  
24 days. The birds were then allocated to 9 dietary treatments (a 3 × 3 factorial arrangement of  
25 treatments) with each treatment replicated 6 times. The factors were three diet types (based on  
26 wheat-soybean meal (WS), maize-wheat-soybean meal (MWS) or barley-wheat soybean meal  
27 (BWS)) and three length of adaptation to dietary treatments (10, 7 or 4 d). The WS was the  
28 references diet whereas MWS and BWS were the assay diets. The adaptation period  
29 corresponded to 10, 7 or 4 days of feeding experimental diets prior to the end of each  
30 experiment. Excreta were collected on the last two days of each experiment. The AME of maize  
31 and barley in the assay diets, in each experiment, were calculated using difference method. On  
32 all the responses considered, there was no significant diet type × adaptation length interaction in  
33 any of the poultry species. Regardless of the poultry species, AME was greater ( $P < 0.05$ ) for  
34 maize compared with barley. The AME (MJ/kg) for maize was 13.5, 13.5 and 13.6 for broilers,  
35 turkeys and laying hens, respectively whereas the corresponding AME (MJ/kg) of barley was  
36 12.2, 11.8 and 12.6, respectively. The effect of adaptation length during AME assay was  
37 statistically significant in turkeys, tended to be significant in broilers but not significant for  
38 laying hens. It was concluded that irrespective of poultry species, the greater overriding factor  
39 affecting AME determined by the difference method is the particular feedstuff being assayed.

40 However, length of adaptation to experimental diets during the assay becomes more important in  
41 birds with relatively physiologically immature digestive tract.

42 *Keywords:* adaptation length, broilers, cereal grains, metabolizable energy, turkeys

43 *Abbreviations:* AME – apparent metabolisable energy; AMEn – nitrogen-corrected apparent  
44 metabolisable energy; BWS – barley wheat soybean meal diet; cEM – coefficient of energy  
45 metabolisability; CF – crude fibre; CP – crude protein; DM – dry matter; MWS – maize wheat  
46 soybean meal diet; N – nitrogen; WS – wheat soybean meal diet.

47

## 48 **1. Introduction**

49 The enactment of various legislations that led to the imposition of ban on some feed  
50 ingredients has drastically reduced the available feedstuff for animal nutrition. For instance, the  
51 abolishment of feed resources of animal origin due to the incidence of transmissible spongiform  
52 encephalopathy in most European countries (Veldkamp and Bosch, 2015). Therefore, the quest  
53 for novel feed resources is currently receiving attention more than ever before. To accurately  
54 compute the nutritive values of these potential ingredients, it is pertinent to investigate the  
55 influence of varying length of adaptation on energy utilisation of feedstuff, in order to determine  
56 the optimum adaptation period in studies involving energy utilisation of currently used and novel  
57 feed ingredients. This becomes imperative in order to comply with the recent advocacy on  
58 precision livestock feeding – which involves feeding of the animals as very close as possible to  
59 their requirements. To achieve this, a careful selection of methodology which gives a precise  
60 availability of energy in an ingredient should be considered. Poultry diets are formulated based  
61 on the AME content and this usually accounts for over 60% of total cost of feeding. So an

62 accurate determination of available energy is essential for optimising the efficiency of the diet  
63 through reduction in feeding cost and excretion of nutrients into the environment.

64 Over the years, several studies have extensively studied the AME of various feedstuffs  
65 (Sibbald, 1975; Sibbald and Price, 1975; Sibbald, 1976; Farrell, 1978; Mollah et al., 1983).  
66 However, one prominent method used in these aforementioned studies was the evaluation of  
67 available energy from digestibility measurement. There are chiefly two procedures in evaluating  
68 digestibility, the total collection method, and the index method (Kong and Adeola, 2014). The  
69 total collection method is laborious and greatly relies on the quantitative measurement of feed  
70 intake and excreta output, whereas the index method depends solely on accurate chemical  
71 analysis of the index compounds. In each of the two methods, the birds are usually adapted to  
72 their cages and feed before sample collection. The adaptation usually lasts for about 3-14 days  
73 (Kong and Adeola, 2014) during which the animal is adjusted to a feeding level to remove extra  
74 work incurred through collection, drying and analysis of orts due to feed refusal by the birds.  
75 However, irrespective of the digestibility procedures, the energy contents of a feedstuff could be  
76 determined by direct, indirect (difference) or regression methods, depending on the contribution  
77 of the basal or test ingredient to the component of interest (Kong and Adeola, 2014).

78 Additionally, several changes occur in the digestive tracts of the birds which may affect  
79 the outcome of the feeding experiments (Zavarize et al., 2012). Feed consumption influenced the  
80 development of digestive tract of birds (Gracia et al., 2003), and duodenum develops earlier than  
81 the jejunum and the ileum (Uni et al., 1999). This affects the rate of nutrient utilisation during the  
82 early stage of life (Uni et al., 1999). However, the intestinal development reached its peak earlier  
83 in broilers compared with laying hens (Zavarize et al., 2012). Most of the experiments involving  
84 energy utilisation are conducted in two phases (Bolarinwa et al., 2012; Adebisi and Olukosi,

85 2015; Kong and Adeola, 2016). The birds are usually fed a basal diet for about 14 days (Adeola  
86 and Ileleji, 2009; Adebisi and Olukosi, 2015) before assay diets are introduced to them. Thus,  
87 there is a feed changeover and consequently a requirement for adaptation to the assay diets prior  
88 to samples collection. The optimum length of the adaptation to the assay diet is not clearly  
89 defined but it is likely to be affected by the nature of the assay feedstuff. Cereals grains are  
90 majorly used as energy sources in poultry diets; however, the difference in their content and type  
91 of fibre often influence their utilisation (JøRgensen et al., 1996). The structural variation in  
92 dietary fibre sources often affect their nutritional, physical and biological properties (Theander et  
93 al., 1989). Therefore, the objective of the current studies is to examine the influence of  
94 adaptation period on the AME content of cereal grains having different fibre content and type.

## 95 **2. Materials and methods**

96 Bird management and sample collection procedures used in the studies were approved, as  
97 appropriate, by Scotland's Rural College Animal Experiment Committee or University of  
98 Kentucky Institutional Animal Care and Use Committee.

### 99 2.1. Animals and management

100 Three hundred and twenty-four Cobb 500 male broiler chicks (Experiment 1) or 162  
101 BUT 10 male turkey poults (Experiment 2) or 162 Lohmann brown laying hens (Experiment 3)  
102 were used for the determination of AME and AMEn contents of maize and barley using the  
103 difference method. The broiler chicks and poults were obtained from local hatcheries and fed  
104 pre-experimental diets for at least 11 days posthatch. The laying hens were obtained at 32 weeks  
105 of age and fed pre-experimental diets for three weeks prior to the start of the experiment. The  
106 pre-experimental diets for all experiments were formulated to meet energy and nutrients  
107 recommendations in accordance with the specific breed requirements. Birds were reared in

108 houses with facilities to regulate temperature, light, and humidity. The birds had *ad libitum*  
109 access to feed and water throughout the pre- and experimental periods. In each experiment, the  
110 birds were allocated to 9 dietary treatments in a randomised complete block design using initial  
111 body weight as blocking criterion. The treatments were in a 3 × 3 factorial arrangement. The  
112 factors were three diet types based on wheat-soybean meal (WS), maize, wheat-soybean meal  
113 (MWS) or barley, wheat-soybean meal (BWS) and three adaptation lengths for adaptation to  
114 dietary treatment (10, 7 or 4 days prior to the end of respective experiments). In all the three  
115 experiments, each treatment was replicated 6 times. However, experiment 1 contained six birds  
116 per replicate, whereas three birds per replicate were used in experiment 2 or 3.

## 117 2.2. Diets and sample collection

118 Three experimental diets were used in each of the three experiments. These diets  
119 composed of a wheat-soybean meal referenced diet, containing no maize or barley, and two  
120 assay diets containing 300 g/kg maize or barley, respectively. Energy yielding ingredients such  
121 as wheat, soybean meal, and soy-oil were substituted with maize or barley in a way their ratios  
122 were similar across all the experimental diets. This was necessary to determine the AME and  
123 AMEn of maize and barley using the difference method. Titanium dioxide (TiO<sub>2</sub>) was added to  
124 all diets (5 g/kg diet) as an indigestible marker for determination of AME content by the index  
125 method. Experiment 1 diets were offered from d 14 to 24, 17 to 24 or 21 to 24 to give 10-, 7- or  
126 4-d adaptation lengths, respectively. Experiments 2 and 3 diets were offered from d 11 to 21, d  
127 14 to 21 or d 17 to 21 which correspond to 10-, 7- and 4-d adaptation lengths, respectively. The  
128 ingredient and chemical composition of the experimental diets used in the three experiments are  
129 shown in Table 1. Excreta were collected on the last two days of each experiment, pooled within  
130 a cage and dried in a convectional oven.

131 2.3. Chemical analysis

132 Samples of diets, wheat, maize, barley, and excreta were ground to pass through 0.5 mm  
133 screen using a mill grinder (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany)  
134 before chemical analysis. Samples of diets, maize, barley and excreta were analysed for N, GE,  
135 DM and TiO<sub>2</sub> where necessary. To determine DM content, samples were dried at 105°C for 24 h  
136 using AOAC method 934.01 (AOAC, 2006) in a drying oven (Uniterm, Russel-Lindsey  
137 Engineering Ltd., Birmingham, England, UK). The analysis of GE was done using a bomb  
138 calorimeter (Parr adiabatic bomb calorimeter, model 6200, parr instruments, Moline, IL, USA)  
139 using benzoic acid as a calibration standard. Titanium dioxide was analysed as described by  
140 Myers et al. (2004) for Experiment 1 or Short et al. (1996) for Experiments 2 and 3. Nitrogen  
141 was determined by the combustion method using AOAC method 968.06 (AOAC, 2006).

142 2.4. Calculations and Statistical analysis

143 Coefficient of nitrogen or energy retention (En) was calculated using the index method as  
144 described in the equation:  $En = 1 - [(Ti/To)X (Eo/Ei)]$ ; where Ti is the concentration of  
145 marker in feed; To is the concentration of marker in the excreta; Eo is the concentration of  
146 energy or N in the excreta, and Ei is the concentration of energy or N in the feed.

147 Metabolizable energy (kcal/g) was calculated using this relation:  $AME = GE_i -$

148  $\left( GE_o \times \left( \frac{Ti}{To} \right) \right)$  where GE<sub>i</sub> and GE<sub>o</sub> are the gross energy (MJ/kg) in feed and excreta,

149 respectively; Ti and To are the concentration of titanium in the diet and excreta, respectively.

150 The cEM of test feedstuffs were calculated using the indirect method after correcting for  
151 the non-energy yielding fractions in the diets as described by Olukosi and Adeola (2009). The



152 test ingredient cEM was calculated using the equation:  $EM_{ti} = \frac{\{EM_{td} - [EM_{rd} \times (1 - FC_{ti/td})]\}}{FC_{ti/td}}$ , where  
153  $EM_{ti}$  is the coefficient of energy metabolizability of the test ingredient,  $EM_{td}$  is the coefficient of  
154 energy metabolisability of the assay diet,  $EM_{rd}$  is the coefficient of energy metabolisability of the  
155 reference diet,  $FC_{ti/td}$  is the fractional contribution of the test ingredient to the assay diet.

156 The AME (MJ/kg) of test feedstuffs was calculated using the equation  $AME_{ti} = EM_{ti} \times$   
157  $GE_{ti}$ , where  $AME_{ti}$  and  $GE_{ti}$  are the metabolisable and gross energy, respectively, of the test  
158 feedstuff.  $EM_{ti}$  is as defined previously. To determine the AMEn, the caloric value of 34.39 kJ/g  
159 was used (Hill and Anderson, 1958).

160 Data were analysed using the Generalised Linear Models of IBM SPSS statistical  
161 package (version 22). Statistical significance was set at  $P \leq 0.05$  for all means comparisons. The  
162 digestibility of the diets (both DM and N), dietary AME and AMEn were analysed as a  $3 \times 3$   
163 factorial of diet type (WS, MWS or BWS) and varying adaptation lengths (10 days, 7 days or 4  
164 days) using ANOVA procedures (presented as supplementary data). Similarly, the AME, AMEn,  
165 and cEM of test ingredients were analysed as a  $2 \times 3$  factorial of cereal grains (maize or barley)  
166 and different adaptation lengths (10 days, 7 days or 4 days) using ANOVA procedures. The main  
167 effects of diets, test ingredients and adaptation lengths and their possible interactions on AME  
168 and AMEn of both diets and cereals, and total tract retention were analysed, and data were  
169 presented with both the main and simple effects.

### 170 3. Results

171 The analysed nutrient composition of wheat, barley, and maize used in the current study  
172 are shown in Table 2. The CP and ash contents in barley and maize were lower compared with

173 wheat. Conversely, the crude fat content in maize and barley were higher compared with wheat.  
174 The CF content in barley was higher compared to wheat which was higher than that of maize.

175 The AME, AMEn and cEM of maize and barley in broilers receiving experimental diets  
176 for different adaptation lengths are presented in Table 3. There was neither significant cereal  
177 grain  $\times$  adaptation length interaction nor significant main effect of adaptation length for AME,  
178 cEM and AMEn. On the other hand, AME, cEM and AMEn were greater ( $P < 0.01$ ) in maize  
179 compared with barley.

180 The AME, cEM and AMEn of maize and barley in turkeys receiving experimental diets  
181 for different adaptation lengths are presented in Table 4. There was no significant cereal grain  $\times$   
182 adaptation length interaction on AME, cEM, and AMEn. The main effect of cereal grain was  
183 significant ( $P < 0.05$ ) on AME, cEM and AMEn in turkeys being greater ( $P < 0.01$ ) for maize  
184 compared with barley. There was significant ( $P < 0.05$ ) main effect of adaptation length on AME  
185 and cEM but not on AMEn. The AME was greater ( $P < 0.05$ ) when determined at 4-d compared  
186 with at 7-d whereas 10-d was intermediate. cEM was greater ( $P < 0.05$ ) at 10-d and 4-d  
187 adaptation lengths compared with at 7-d adaptation length.

188 The AME, cEM and AMEn of maize and barley in laying hens receiving experimental  
189 diets for different adaptation lengths are presented in Table 5. There was no significant cereal  
190 grain  $\times$  adaptation length interaction ( $P > 0.05$ ) nor significant main effect of adaptation length  
191 for AME, cEM and AMEn. Conversely, the main effect of cereal grain was significant for AME,  
192 cEM and AMEn. The AME, cEM and AMEn were greater ( $P < 0.05$ ) in maize compared with  
193 barley.

#### 194 **4. Discussion**

195           The current experiments were carried out to investigate the impact that varying lengths of  
196 adaptation length during AME assay, using different poultry species, could have on AME value  
197 of two cereal grains with different fibre profiles.

198           The nutrient composition of wheat, barley, and maize used in this current study are  
199 comparable with values reported previously (Lin et al., 1987). The results obtained in the current  
200 study shows that irrespective of the species of poultry birds, increasing the fibre contents of the  
201 diet (via test ingredient) results in lower metabolisability and consequently lower AME content.  
202 Furthermore, high-fibre feedstuff led to lower total tract DM and N retention. These could be  
203 attributed to limited amounts of endogenous enzymes necessary to enhance release of available  
204 energy in the cereals grains.

205           Masey O'Neill et al. (2014) noted that starch supplies the majority of the available energy  
206 in cereals grains, however, this is encapsulated in the cell wall which makes it inaccessible to  
207 poultry birds due to their inability to produce sufficient amounts of endogenous enzymes in  
208 hydrolysing plant materials (Bedford, 1995). Maize contains more starch than barley. Depressed  
209 starch digestibility lower metabolisable energy content of cereals (Mollah et al., 1983; Rogel et  
210 al., 1987). Additionally, high fibre feedstuffs like barley contain NSP which have been reported  
211 to increase the viscosity of the digesta in poultry. The presence of B-glucans in barley primarily  
212 increase the viscosity of the digesta (Burnett, 1966). Increased viscosity could reduce the  
213 digestibility and availability of nutrients to the animals (Bedford, 1995; Choct et al., 1996;  
214 Masey O'Neill et al., 2014). The increased viscosity and bulk of intestinal content impede the  
215 rate of diffusion of substrate and consequently hinder their effective interaction at the mucosal  
216 surface (Choct et al., 1996).

217           Additionally, the viscosity of digesta could decrease DM intake due to the increase in  
218 satiety value. Further calculations in the current study showed that the DM intake of birds on  
219 maize-based diets was higher compared with the other two diets. Dietary fibre may lower N  
220 retention in poultry birds (Janssen and Carré, 1985; Mateos et al., 2012). As expected, the total N  
221 retention of broilers decreased with increasing fibre level in the diet. With the highest total N  
222 retention observed in MWS compared with WS and BWS. The NSP content of cereals reduced  
223 protein digestibility (Choct and Annison, 1992). On the other hand, increasing the fibre content  
224 of the diet through addition of wheat and barley as oppose to maize did not decrease total N  
225 retention in laying hens and turkeys. Laying hens retained more N in their body compared to  
226 broilers and turkeys.

227           The neutral detergent fibre (NDF) content in barley was nearly three-fold that recorded in  
228 maize, which is an indication of the level of fibre present in the two test ingredient. The  
229 determined AME (MJ/kg) of barley for broilers (12.2) and laying hens (12.6) in this experiment  
230 correspond with the values reported by Choct et al. (2001). However, these values were slightly  
231 higher than that observed in turkeys. This variation could be associated with the difference in the  
232 physiological age of the birds. Although, turkeys and broilers used in this current experiment  
233 were of similar age but broilers have more physiologically matured digestive tract than turkeys.

234           Likewise, adults laying hens were used in this bioassay. Adults birds have the ability to  
235 utilise NSP more effectively, thus the higher the NSP level in the grain, the larger the difference  
236 in metabolisable energy (Choct et al., 2001). Choct et al. (1996) noted that NSP depress the  
237 AME content of feedstuff due to their tendency to inhibit digestion of starch, protein and lipid.  
238 The AME content of maize reported for the three poultry species are similar and this could be  
239 traced to the less viscous nature of the grain. Choct et al. (2001) argued that the variation in

240 AME content of cereals in young and mature birds could as well be due to the degree of viscosity  
241 of the grain. With the effect more pronounced in high viscous grain (barley) than less viscous  
242 ones like maize.

243 In order to acclimatise the birds to a specific feedstuff or eliminate the possible  
244 confounding effects of changing over feeds (from pre-experiment diet to test, or assay, diets) in  
245 experiments, it is common practice to adapt the experimental animals to test ingredients or feeds  
246 for a certain number of days followed by the actual feeding and samples collection periods.  
247 There seems to be no consensus on the actual number of days to be used as adaptation period.  
248 During this period, several changes occur in the digestive tract which may eventually influence  
249 the results of the study. Adaptation length varies from 3-14 days in most experiments. Steinfeldt  
250 et al. (1998) employed 3 days of adaptation when determining the digestibility and AME of  
251 wheat-based diet in broilers. Hew et al. (1998) and Adebisi and Olukosi (2015) utilised 7 days  
252 of adaptation in determining the AME content of wheat and wheat-DDGS respectively, in  
253 broilers. 10-d adaptation period was used by Cowieson and Ravindran (2008) in investigating the  
254 effect of exogenous enzymes on energy digestibility of maize-based diets in broilers. Likewise,  
255 conventional grower diet was fed for 10-d adaptation period in determining the influence of  
256 enzyme supplementation on metabolisable energy of solvent extracted rapeseed and sunflower  
257 seed meal in chicken, quail and guinea fowl (Mandal et al., 2005). It is possible however, that  
258 adaptation period required may differ depending on fibre type and content of test feedstuff or on  
259 the age of the birds or their combination.

260 The data from the current experiment suggest that adaptation periods influenced the  
261 metabolisable energy content of cereal grain and assay diets more markedly in turkeys but to a  
262 less extent in broilers and hardly at all in laying hens. However, the effect of adaptation period

263 on AME content of cereal grain in turkeys was inconsistent with shortening length of adaptation,  
264 as the highest determined AME was observed with 10-d and 4-d adaptation period compared  
265 with 7-d. In broilers, metabolisable energy content of cereal grain and assay diets only  
266 marginally reduced with decreasing length of adaptation period. The determined metabolisable  
267 energy of cereal grain and assay diets was similar for the three different adaptation periods in  
268 laying hens.

269         The differences in physiological maturity among the different birds used in the current  
270 experiment may partly explain why there were differences in the effect of adaptation length  
271 during AME assay in broilers, turkey and laying hens. The data from the current study indicate  
272 that the effect of varying length of adaptation was more apparent in birds with less matured  
273 digestive system, namely broiler and turkeys, rather than the laying hens. Turkeys are likely less  
274 physiologically mature at the same age with broilers in view of their different rates of growth as  
275 has been demonstrated between turkeys and duck (Applegate et al., 2005). This may explain why  
276 the effect of adaptation length was clearly significant in turkey, tended to be significant for  
277 broilers but not significant for laying hens. Uni et al. (1995) demonstrated that villi height and  
278 width increased 25 to 100% in all segment of small intestine between 4 and 10 d of age.  
279 Additionally, digestive enzymes may be limiting for adequate nutrient utilisation posthatch in  
280 chick (Nitsan et al., 1991) and poults (Sell et al., 1991) and these enzymes concentration increase  
281 with age. Therefore, during the development and maturation of the digestive system in young  
282 poultry, dietary nutrients may be poorly utilised, most especially during the first ten days after  
283 hatching (Batal and Parsons, 2002).

284         The lowest AME values was observed between 4 and 7 d post-hatching in chicks  
285 (Zelenka, 1968) and turkeys (Sell, 1996) and this later increased with age. Siregar and Farrell

286 (1980) found that AME values were not affected by age, whereas Bartov (1988) reported a  
287 decrease in AME with birds grew older. Similarly, AMEn value increased from d 0 to d 14 for  
288 New Hampshire × Columbian chicks whereas the increase lasted until d 21 of age with the  
289 commercial chick (Batal and Parsons, 2002). Also, there was evidence that AME and AMEn  
290 varied with age of birds in the study of Lopez and Leeson (2008). There is also the possibility  
291 that stability in the microbial population of the digestive tract with age (Lu et al., 2003)  
292 influences how birds at different ages respond to the influence of adaptation length on AME of  
293 feedstuffs. The variation in energy content of a single feedstuff determined at different period  
294 after hatching corroborate the findings in this experiment where adaptation period influenced the  
295 metabolisability of energy in cereal grains and assay diets in turkeys and broilers but not in  
296 laying hens.

## 297 **5. Conclusions**

298 The results of the current study show that irrespective of the species of poultry birds, the  
299 greater overriding factor for energy values of feedstuff is the specific ingredient being assayed.  
300 However, the influence of adaptation period during AME assay becomes relevant when the assay  
301 utilises birds with relatively physiologically immature digestive tract.

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417

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425 **Table 1**

426 Ingredient and chemical composition (g/kg) of experimental diets to determine apparent metabolisable energy content of maize and barley fed for  
 427 different adaptation lengths in broilers, turkeys and laying hens.

Item	Broilers			Turkeys			Laying hens		
	1	2	3	1	2	3	1	2	3
Items									
Wheat	602.2	411.9	411.9	492.8	333.4	333.4	654.1	433.4	433.4
Soybean meal	300.0	205.2	205.2	400	270.7	270.7	210	139.1	139.1
Soy oil	47.0	32.2	32.2	35.0	23.7	23.7	25.0	16.6	16.6
Dicalcium phosphate	17.5	17.5	17.5	26	26	26	9.5	9.5	9.5
Limestone	14.0	14.0	14.0	17.7	17.7	17.7	85.0	85.0	85.0
Titanium dioxide <sup>a</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Maize	0	300	0	0	300	0	0	300	0
Barley	0	0	300	0	0	300	0	0	300
Vitamin-mineral premix <sup>b</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
L-Lysine HCl	3.6	3.6	3.6	8.0	8.0	8.0	1.0	1.0	1.0
DL-Methionine	1.9	1.9	1.9	3.9	3.9	3.9	1.4	1.4	1.4
L-threonine	0.7	0.7	0.7	2.6	2.6	2.6	0.8	0.8	0.8
Salt (NaCl)	3.1	3.1	3.1	4.0	4.0	4.0	3.2	3.2	3.2

Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Analysed energy and nutrient composition <sup>c</sup>									
Dry matter, g/kg	898.9	896.9	903.1	892.2	894.4	894.1	893.0	896.8	896.8
Gross energy, MJ/kg	17.4	17.0	17.0	16.4	15.9	15.8	14.7	14.6	14.6
Crude protein (N x 6.25), g/kg	240.6	195.0	199.4	253.1	195.6	206.3	182.5	137.5	142.5

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429 <sup>a</sup> Prepared as 1g titanium dioxide added to 4g of ground corn.

430 <sup>b</sup> Vitamin A, 5484 IU; vitamin D3, 2643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg, d-panthothenic acid, 11 mg; niancin,  
431 44.1 mg, choline chloride, 771 mg; vitamin B12, 13.2 ug; biotin, 55.2 ug; thiamine mononitrate, 2.2 mg; folic acid, 990 ug; pyridoxine hydrochloride, 3.3 mg;  
432 iodine, 1.11 mg; manganese, 66.06 mg; copper, 4.44 mg; iron, 44.1 mg; zinc, 44.1 mg; selenium, 300 ug.

433 <sup>c</sup> Values are means of duplicate analyses.

434 **Table 2**  
 435 Analysed chemical composition of wheat, barley and maize (g/kg, as-is basis).

Item	Wheat	Barley	Maize
Dry matter	882.3	892.9	871.9
Crude protein (N x 6.25)	159.9	109.3	84.4
Gross Energy (MJ/kg)	-	17.9	18.1
Crude fat	11.7	47.4	31.2
Crude fiber	21.7	38.8	15.2
Ash	19.7	15.6	13.0
Acid detergent fiber	41.1	67.9	23.6
Neutral detergent fiber	273.1	186.5	68.5

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451 **Table 3**

452 Apparent metabolisable energy, coefficient of energy metabolisability and nitrogen-correct  
 453 metabolisable energy of maize and barley in broilers receiving experimental diets for different  
 454 adaptation lengths<sup>1</sup>.

Cereal grain	Adaptation length	AME (MJ/kg)	cEM	AMEn (MJ/kg)
Means for main effect of cereal grain				
Maize		13.5	0.747	13.4
Barley		12.2	0.685	12.1
Pooled SEM <sup>2</sup>		0.233	0.013	0.240
P-values of diet type		0.001	0.003	0.001
Means for main effect of adaptation length				
	10 days	13.3	0.740	13.2
	7 days	13.0	0.720	12.8
	4 days	12.4	0.688	12.2
Pooled SEM <sup>2</sup>		0.285	0.016	0.295
P-values of adaptation length		0.089	0.093	0.062
Means for simple main effects				
Maize	10 days	13.9	0.766	13.8
	7 days	13.5	0.749	13.5
	4 days	13.1	0.727	13.0
Barley	10 days	12.8	0.713	12.7
	7 days	12.4	0.691	12.2

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	4 days	11.6	0.650	11.4
Pooled SEM <sup>2</sup>		0.403	0.023	0.417
Cereal grain × adaptation length		0.865	0.826	0.885

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455 <sup>1</sup>n of 6 for the simple effect, 18 for main effect of adaptation length and 27 for main effect of  
456 cereal grain. AME = Apparent metabolisable energy; cEM = Coefficient of energy  
457 metabolisability; AMEn = Nitrogen-corrected apparent metabolisable energy.  
458 <sup>2</sup>SEM = Standard error of difference of mean.

459

460 **Table 4**

461 Apparent metabolisable energy, coefficient of energy metabolisability and nitrogen-correct  
 462 metabolisable energy of maize and barley in turkeys receiving experimental diets for different  
 463 adaptation lengths<sup>1</sup>.

Cereal grain	Adaptation length	AME (MJ/kg)	cEM	AMEn (MJ/kg)
Means for main effect of cereal grain				
Maize		13.7	0.756	13.3
Barley		11.6	0.649	11.2
Pooled SEM <sup>2</sup>		0.340	0.019	0.369
P-values of diet type		0.001	0.002	0.002
Means for main effect of adaptation length				
	10 days	12.9 <sup>ab</sup>	0.716 <sup>a</sup>	12.7
	7 days	11.7 <sup>b</sup>	0.651 <sup>b</sup>	11.4
	4 days	13.3 <sup>a</sup>	0.741 <sup>a</sup>	12.8
Pooled SEM <sup>2</sup>		0.446	0.025	0.491
P-values of adaptation length		0.046	0.013	0.073
Means for simple main effects				
Maize	10 days	14.4	0.795	14.2
	7 days	12.7	0.703	12.4
	4 days	13.9	0.771	13.4
Barley	10 days	11.4	0.637	11.2
	7 days	10.7	0.600	10.3

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	4 days	12.7	0.710	12.1
Pooled SEM <sup>2</sup>		0.552	0.031	0.736
Cereal grain × adaptation length		0.417	0.330	0.371

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464 <sup>1</sup>n of 6 for the simple effect, 18 for main effect of adaptation length and 27 for main effect of  
465 cereal grain. AME = Apparent metabolisable energy; cEM = Coefficient of energy  
466 metabolisability; AMEn = Nitrogen-corrected apparent metabolisable energy.

467 <sup>2</sup>SEM = Standard error of mean.

468 <sup>a-b</sup> Means in the same column but with different superscripts are significantly different (P <  
469 0.05).

470

471 **Table 5**

472 Apparent metabolisable energy, coefficient of energy metabolisability and nitrogen-correct  
 473 metabolisable energy of maize and barley in laying hens receiving experimental diets for  
 474 different adaptation lengths<sup>1</sup>.

Cereal grain	Adaptation length	AME (MJ/kg)	cEM	AMEn (MJ/kg)
Means for main effect of cereal grain				
Maize		13.6	0.751	13.5
Barley		12.6	0.703	12.5
Pooled SEM <sup>2</sup>		0.247	0.014	0.282
P-values of diet type		0.022	0.017	0.029
Means for main effect of adaptation length				
	10 days	13.2	0.734	13.2
	7 days	12.6	0.702	12.5
	4 days	13.4	0.746	13.4
Pooled SEM <sup>2</sup>		0.312	0.017	0.354
P-values of adaptation length		0.271	0.164	0.267
Means for simple main effects				
Maize	10 days	13.5	0.745	13.4
	7 days	13.2	0.727	13.0
	4 days	14.1	0.777	14.0
Barley	10 days	12.9	0.722	12.9
	7 days	12.1	0.676	12.0

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	4 days	12.8	0.714	12.7
Pooled SEM <sup>2</sup>		0.437	0.002	0.503
Cereal grain × adaptation length		0.822	0.740	0.799

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475 <sup>1</sup>n of 6 for the simple effect, 18 for main effect of adaptation length and 27 for main effect of  
476 cereal grain. AME = Apparent metabolisable energy; cEM = Coefficient of energy  
477 metabolisability; AMEn = Nitrogen-corrected apparent metabolisable energy.  
478 <sup>2</sup>SEM = Standard error of mean.

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