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1	ADAPTATION TO ALTERNATIVE DIETS IN BROILERS
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### 21 ABSTRACT

22 Poultry production currently relies on the use of soybean as the main protein and energy source. Reducing its proportion in poultry diets and partly replacing it with local feedstuffs 23 would improve sustainability by reducing dependence on importations and the environmental 24 impact of production. In this study, we evaluated the impact of replacing soybean by 25 sunflower meal, fava bean, canola meal, and dried distillers' grains with solubles on the 26 performance of rapid- and slow-growing chickens. Animals were reared in groups and on the 27 floor. Individual body weight and feed intake data were collected throughout each animal's 28 life thanks to an electronic feed station. At 5 weeks (for broilers) and 12 weeks (for slow-29 30 growing chickens), the birds were slaughtered to obtain carcass composition and meat quality data. 31

Adaptation to the alternative diet was studied separately for each genotype. Firstly, we did ANOVA with diet effect on daily data of individual body weight, feed intake, and feed conversion ratio. Secondly, the variability of performances within the group was studied by ANOVA with effects of diet, period and their interaction. Finally, the correlations between daily performances and final performances at slaughter were calculated to understand the construction of final phenotypes and to identify early indicators of final performances.

38 The results first showed that the animals adapted well to the alternative diet, mean daily and final performances being mostly similar between the two diets for both genotypes (<3% on 39 final BW). However, daily observations highlighted the critical importance of periods around 40 dietary transitions by showing impacted performances for both genotypes. For example, FCR 41 of LR-AD was 12 to 14% lower during the three days after transitions than during the three 42 days before. It underlined the fact that adapting management of the batch to the alternative 43 diet would be necessary. Correlations between daily and final performances showed that the 44 slaughter performances of rapid-growing chickens were mostly determined by body weight 45

whereas the main criterion was cumulative feed conversion for slow-growing chickens. These
correlations also suggested that reserve making might be modified with the alternative diet,
with rapid-growing chickens making more glycogen reserves and less fat reserves.

# Key Words: alternative feedstuff, Radio frequency identification device, kinetics, feed efficiency, feed intake

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#### 52 INTRODUCTION

Nutrition represents 50 to 70% of the production costs in poultry production (van Horne, 53 2018). A large part of these costs comes from the reliance on soybean and cereals to feed 54 animals, which often compete with human nutrition (Leinonen and Kyriazakis, 2016). In 55 Europe, soybean is mostly imported from America (European Commission, 2019). Moreover, 56 Lathuillière et al. (2017) reported that soybean is a major cause of deforestation in Brazil and 57 58 that maize culture requires a large amount of water. There is thus a motivation to reduce the need of these two feedstuffs in poultry diets in order to ensure the sustainability of poultry 59 production in a context of growing world demand. Sunflower and rapeseed meals, by-60 products of oil industry and Dried Distillers Grains with Solubles (DDGS), by-product of 61 bioethanol production, can be used as alternative sources of proteins. Moreover, their protein 62 63 content varies according to the method of production (Laudadio et al., 2013). In order to compensate a potential lack of protein, other sources can be added to the diet, such as fava 64 bean, a legume rich in protein with a sustainable worldwide production (Jensen et al., 2010). 65 However, their incorporation is limited due to these beans richness in protease inhibitors, 66 67 lectins, phenolic compounds, saponins and non-starch polysaccharides that can affect the feed efficiency of the animals by impacting transit time, nutrient degradation or anatomy of the 68 digestive tract for example (Diaz et al. 2006). It has been shown that replacing soybean by a 69 unique feedstuff had negative consequences on performances. For example, replacing 70

soybean by fava bean led to low digestibility in methionine and cysteine (Koivunen et al, 71 72 2016). Regarding performances, replacing soybean by fava bean led to a decrease of 3% to 9% in BW with an increase of 5.7 to 8.0% of FCR in standard and Label Rouge chickens 73 (Diaz et al., 2006; Bosco et al., 2013). Replacing it by DDGS improved BW by 2.1% to 3% 74 without modifying FCR (Foltyn et al., 2013). Finally, replacing by canola meal increased 75 FCR by 1% due to a 7.1% decrease of BW and DFI (Toghyani et al., 2016). Taking into 76 account these results, one nutritional strategy could be using a mixture of these alternative 77 feedstuffs (sunflower and canola meals, DDGS, fava beans) instead of a unique feedstuff, 78 assuming that the complementarity between feedstuffs and the limitation of the proportion of 79 80 each anti-nutritional factor would favor bird adaptation.

We thus evaluated the ability to adapt of two genotypes with different levels of growth rates 81 and nutritional requirements, i.e. rapid-growing standard chickens and slow-growing Label 82 Rouge chickens. We compared the kinetics of mean body weight, feed intake, and feed 83 efficiency, as well as the variability of these traits between the alternative and the control diet 84 85 from hatch to slaughter. Finally, the analysis of the profiles of correlations between daily data and carcass and meat quality traits measured at slaughter was used to decipher how final 86 phenotypes were constructed in both genotypes and diets and to find early predictors, other 87 88 than morphological traits such as chicks or chickens' length and weight (Mendes et al., 2007; Moleenar et al., 2009). Measuring these traits in animals reared in individual cages induces a 89 bias as it modifies animal feeding behavior and physical activity. Collective performances 90 collected from birds reared in floor pens do not have this bias, but require a large number of 91 animals for a rather poor statistical power (Alagawany et al., 2017; Gopinger et al., 2014). In 92 93 order to be representative of production conditions (i.e. with animals reared on the floor and in groups), we thus collected individual feed intake and body weight data with an automaton 94 developed in our lab. 95

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#### 98 MATERIALS AND METHODS

99 The present study was performed in agreement with the French National Regulation for 100 human care and use of animals for research purposes and received the authorization number 101 2018062715076382.V2-15695. Animals were reared at the PEAT INRAE poultry 102 experimental facility (2018, https://doi.org/10.15454/1.5572326250887292E12) registered by 103 the French Ministry of Agriculture under license number C-37-175-1 for animal 104 experimentation (INRAE, Centre Val de Loire, and Nouzilly, France).

#### 105 Birds and Housing

Two batches of animals were reared successively for this experiment. In the first batch, 80 106 male SASSO naked neck chickens, a slow-growing genotype dedicated to Label Rouge 107 production (LR) were reared from 1 to 82 days, between September and December 2018. In 108 109 the second batch, 80 Cobb 500 male chickens (STD) were reared from 1 to 35 days, between January and February 2019. Lighting and temperature schedules for both genotypes are 110 provided in Supp. Table 1. At 1 day of age, the animals were identified with a wing band and 111 112 an electronic Radio frequency identification device (RFID) chip, then weighed and placed in one pen on a floor covered with wooden chips. The RFID chip was placed at the base of the 113 neck and secured with a plastic string passing under the skin. The pen was divided into two 114 parts by a mesh bulkhead and the animals were dispatched into one of the two groups, with an 115 equal starting weight for both groups. In the first part, the animals were fed with a classic 116 117 corn-soybean diet (CD) as used in usual commercial conditions. In the second part, the animals were fed with an alternative diet (AD) including less soybean meal and a higher 118 proportion of alternative feedstuffs such as sunflower, rapeseed, and fava bean. The 119

composition of the diets is shown in Table 1. Within a genotype, the diets were isoproteic and 120 isoenergetic. The diets differed between the two genotypes in order to fulfill the needs of 121 slow- or fast-growing broilers. A starter diet was given from hatch to 7 d for STD birds 122 (2850 kcal.kg<sup>-1</sup> DM; 21.5 % CP) and to 28 d for LR birds (2750 kcal.kg<sup>-1</sup> DM; 20.0 % CP). A 123 grower diet was given from 8 to 22 d for STD chickens (2900 kcal.kg<sup>-1</sup> DM; 20.0 % CP) and 124 from 29 to 63 d for LR chickens (2850 kcal.kg<sup>-1</sup> DM; 18.0 % CP). A finisher diet was given 125 from 23 to 35 d for STD chickens (2950 kcal.kg<sup>-1</sup> DM; 18.5 % CP) and from 69 to 82 d for 126 LR chickens (2900 kcal.kg<sup>-1</sup> DM; 16.5 % CP). 127

#### Feed Station 128

Body weight and feed intake were individually and continuously recorded throughout the 129 experiment thanks to an electronic feed station (https://www.feed-a-gene.eu/media/bird-e-130 automate-de-consommation-alimentaire-pour-volailles). The feeder has a circular shape and 131 consists of 8 independent accesses to feed, without corridors, so that the chickens can express 132 133 their natural feeding behavior. Each access includes one feed tube, one feed trough, one antenna placed on the top of the feed trough to detect the animal's RFID chip, one scale for 134 feed weight, and one scale to record animal weight placed under the tray on which the animal 135 climbs to eat. The feed troughs and the trays can be changed according to the size of the 136 animals. Raw data obtained from the station are 1) feed weight by access every second, 2) 137 identity of animal, time and access number every time an antenna detects a chip, and 3) mean 138 animal weight during each visit. A visit is defined by consecutive readings of the same chip at 139 the same access with less than 10 seconds between consecutive detections of the chip. All 140 scales and antennas are connected to a central system of data acquisition. Because of 141 electronic problems, data were acquired from 12 days on for the LR chickens. Reliable data 142 could be obtained from day 3 onward for the STD chickens. 143

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#### 145 Meal Definition and Calculation of Feed Intake per Meal

146 Consecutive visits were grouped into meals as follows. A meal started each time a new chip is detected and ended when another one was read or when the chip was no longer detected 147 during an interval of two minutes. This limit was defined using preliminary experiments 148 during which we compared the behavior of animals obtained by video recording and data 149 coming from the station (unpublished data). Occasionally, the chip is not detected by the 150 antenna immediately after an animal's arrival or that the signal is lost before an animal's 151 departure. In order to correct for this bias, we calculated the variance of feed weight data by 152 intervals of 10 seconds before the start and after the end of the meal. Video analyses showed 153 154 that a large variance of feed weight in the station (>0.1 g) is associated with pecking movement in the feed trough, and thus, that an animal is eating. Meal length was extended to 155 include these periods of large variance. 156

For meal n starting at second  $S_n$  and ending at second  $E_n$ , feed intake (FI<sub>n</sub>) is calculated as the difference of mean feed weight recorded every second between meals n-1 and n and between meals n and n+1. Outlier values of feed weights in these intervals were removed using the Cook's distance with a threshold of 1/k, where k is the number of values in the interval. Feed intake of the meal was obtained as:

$$FI_{n} = \frac{1}{1 + S_{n} - E_{n-1}} \sum_{i=1+E_{n-1}}^{S_{n}-1} FW_{i} - \frac{1}{1 + S_{n+1} - E_{n}} \sum_{i=1+E_{n}}^{S_{n+1}-1} FW_{i} FI_{n}$$
$$= \frac{1}{1 + S_{n} - E_{n-1} - NOV_{1}} \sum_{i=1+E_{n-1}}^{S_{n}-1} C_{i} \times FW_{i}$$
$$- \frac{1}{1 + S_{n+1} - E_{n} - NOV_{2}} \sum_{i=1+E_{n}}^{S_{n+1}-1} C_{i} \times FW_{i}$$

where  $FI_n$  is the feed intake for meal n,  $FW_i$  the feed weight at second i,  $S_n$  and  $S_{n+1}$  the times at which meals n and n+1 start,  $E_{n-1}$  and  $E_n$  the times at which meals n-1 and n end,  $C_i$  a coefficient equal to 0 if the feed intake value at second i was an outlier and 1 if not, NOV<sub>1</sub> and NOV<sub>2</sub> the number of outlier values removed between meals n-1 and n and between means n and n+1, respectively.

When less than 10 seconds separated two successive meals M1 and M2 of respective durations D1 and D2, we did not obtain enough stable feed weight values to calculate a reliable mean feed weight between M1 and M2. A total feed intake ( $FI_{M1M2}$ ) was calculated as the difference between mean feed weight before the start of M1 and after the end of M2. The feed intake of each meal ( $FI_{M1}$ ,  $FI_{M2}$ ) was then calculated according to the respective duration of each meal as:

$$FI_{M1} = \frac{D1}{D1 + D2} \times FI_{M1M2}$$

$$FI_{M2} = \frac{D2}{D1 + D2} \times FI_{M1M2}$$

In order to check the reliability of feed intake measured by the station, each time the feed tubes were refilled, the added quantity of feed was weighed and compared with the data obtained from the feed station after refilling.

The daily feed intake (DFI) was calculated as the sum of the feed intake of all meals eatenduring a 24-hour period.

#### 178 Body weight and daily gain calculation

Before calculating individual body weight (BW) on the different days, abnormal data were removed (weights below 25 g and above three times the mean BW of the previous day). Data outside the interval deviating from the mean by more than three standard deviations were then

removed. Body weight (BW) was then calculated as the mean of all available weight data 182 183 during a day for each animal.

184 In order to check the reliability of animal weight data from the station, animals were weighed manually, weekly for standard chickens and every two weeks for Label Rouge chickens.

#### Average Daily Gain and Feed Conversion Ratio Model 186

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In order to smooth the daily variations of FCR, a moving average was used to calculate the 187 daily feed conversion ratio, as already done in pigs (Huynh-Tran et al., 2017). Among the 188 different possibilities tested, a moving average daily gain over 5 days (ADG) led to the lowest 189 number of null or negative FCR values and the lowest daily coefficient of variation of FCR 190 among individuals. Daily FCR was thus calculated as: 191

$$ADG_{ij} = \frac{BW_{i(j+2)} - BW_{i(j-2)}}{5}$$

$$DFCR_{ij} = \frac{DFI_{ij}}{ADG_{ij}}$$

with BW<sub>ij</sub> being the mean weight of the animal i on day j and DFI<sub>ij</sub> the daily feed intake of 192 animal i for day j. 193

#### Cumulative feed conversion ratio 194

195 The daily cumulative feed conversion ratio for animal i on day j (DCFCR<sub>ii</sub>) was calculated as the ratio of cumulated feed intake between the first day of data collection and day j to the 196 weight gain over the same period: 197

$$DCFCRij = \frac{\sum_{k=1}^{k=j} DFI_{ik}}{BW_{ii} - BW_{i1}}$$

with  $DFI_{ik}$  being the daily feed intake of animal i for day k and  $BW_{ij}$  the body weight of animal i on day j.

200 Carcass Composition and Meat Quality

At 35 d for STD and 82 d for LR chickens, the animals were weighed after 8 hours of feed withdrawal and transferred to the slaughterhouse of the PEAT INRAE poultry experimental facility (2018, https://doi.org/10.15454/1.5572326250887292E12).

204 After 24 hours of chilling, body composition was characterized by measuring breast meat yield (BMY), Pectoralis major yield (PMY), Pectoralis minor yield (PmY), abdominal fat 205 yield (AFY) and thigh yield (TY) in relation to body weight (BW). Except for the abdominal 206 207 fat which was taken entirely, only the right part of the animals was taken and the weight of the different parts was doubled to obtain those yields. Meat quality was evaluated on the 208 Pectoralis major muscle by measuring lightness (L\*), yellowness (b\*) and redness (a\*) of the 209 210 meat with a miniscan spectrocolorimeter (Hunterlab, Reston, VA, USA) and ultimate pH (pHu) with a portable pH meter (model 506, Crison Instruments SA, Alella, Barcelona, 211 212 Spain).

### 213 Analysis of Variance

Analyses were done separately for each genotype, since the experiments had been conducted 214 215 independently. The effect of the diet was first estimated separately for each day by applying the PROC ANOVA procedure of SAS 9.4 (2013) with diet as the single fixed effect to data 216 calculated for each day: body weight (BW), average daily gain (ADG), feed intake (DFI), 217 feed conversion ratio (DFCR), and cumulative feed conversion ratio (DCFCR). In a second 218 step, three rearing phases were defined according to the feeding period: starter (S), grower 219 220 (G), and finisher (F) phases when the animals were fed with the starter, grower, and finisher diets, respectively. The birds' response to the diet depending on the feeding period was then 221 222 analyzed with the following ANOVA model:

$$y_{ijk} = D_i + P_j + DP_{ij} + e_{ijk}$$

with  $y_{ijk}$  being the trait for animal k with diet i and period j,  $D_i$  the fixed effect of diet i,  $P_j$  the fixed effect of phase j (i.e. starter diet, grower diet, and finisher diet),  $DP_{ij}$  the interaction between diet i and phase j and  $e_{ijk}$  the residual for animal k. Both individual daily phenotypes and their coefficients of variation (calculated within-day) were analyzed, in order to consider the birds' response in term of mean and variability.

Diet effect on slaughter traits was estimated by one-way ANOVA within each genotype, dietbeing the only fixed effect of the model.

### 231 Correlations with Daily Data

Correlations between the daily data (BW, ADG, DFI, DFCR) and the data measured at
slaughter (final BW and final cumulative feed conversion ratio (CFCR<sub>f</sub>), BMY, PMY, PmY,
AFY, TY, L, a\*, b\* and pHu) were calculated by using the Rcorr function of the package
Hmisc of the R software (R Core team, 2017).

236

#### 237 **RESULTS**

#### 238 Validation of Growth and Feed Intake Data

On average, the absolute value of the difference between manual and automatically recorded data of body weight was low (2.2%). Similarly, the difference between feed weight displayed by the feed station and the real feed weight at each refilling was low (0.3%).

#### 242 Diet effect on growth parameters

243 *Effect on the mean* The ADG of LR chicken showed the same trends of kinetics in both diets,

with a first phase of increasing, followed by a plateau and a last phase of decreasing (Fig. 1c).

As the length of the plateau lasted 10 days with the AD diet and 20 days with the CD diet,

ADG decreased earlier with AD (after 55 d) than with CD (after 70 d). The ADG of animals 246 247 fed with the AD diet was 8 to 28% higher between 15 and 33 d (starter phase and start of grower phase), and 8 to 45% higher between 48 and 57 d (grower phase). In contrast, from 60 248 to 68d (finisher phase), animals fed with AD showed a 10 to 40% lower ADG than with the 249 CD diet (Supp. Table 3). This kinetics was consistent with a slight advantage of BW for birds 250 fed AD from 14 to 40 days and from 49 to 61 days (4.3 to 8.5%) and the absence of difference 251 after this age (Fig. 1a, Supp. Table 2). Unlike the LR chickens, ADG increased until the end 252 of the experiment for both diets in STD chickens (Figure 1d), which are slaughtered at a much 253 younger age than LR chickens. Diet had a much smaller impact in STD than in LR chickens, 254 255 as shown by the global analysis by feeding period in which diet effect on ADG and BW was significant in STD chickens, but not in LR chickens (Table 2). Only during a 5-days period 256 between 27 and 31 d was ADG 5 to 15% higher with AD than with CD (Supp. Table 3). 257 258 Consistent with the absence of difference in ADG between diets, the growth curves of STD birds were similar between the two diets (Fig. 1b). 259

260 Effect on the variability. The CV for ADG in STD chickens and for BW in both genotypes was stable and low at all ages, usually lower than 20% (Fig. 1a, 1b, 1d). In contrast, CV for 261 ADG in LR chickens varied with age for both diets, being stable until 35 days and increasing 262 263 from 35 to 82 d up to values as high as 50% (Fig. 1c). Despite similar trends, the kinetics of the CV of ADG during the three periods differs between the two diets. For the AD diet, CV 264 increased from the starter to the grower diets while the increase occurred between the grower 265 and finisher phases for the CD diet (Table 3). A significant interaction between diet and phase 266 was also observed on BW variability in LR chickens. Indeed, LR animals fed with AD were 267 268 14.3% less variable than those fed with CD, only during the grower phase, whereas STD chickens fed with AD showed a 27.1% higher variability than those fed with CD over the 269 270 whole period (Table 3).

#### 271 Diet effect on feed intake and efficiency traits.

272 *Effect on the mean.* For both diets and genotypes, as expected, DFI increased with age (Fig. 2). No difference was observed between diets in LR chickens, except at 20, 26, 28 and 42 273 274 days, with no clear advantage for CD or AD (Fig. 2a, Supp. Table 4). In contrast, in STD chickens, DFI was continuously higher with AD than with CD, but the difference was 275 significant only during the 4th week, before the last diet change (Fig. 2b, Supp. Table 4). 276 277 During this period, DFI was 7.4 to 12.4% higher with AD than with CD. Summarizing information by feeding period, we observed a diet effect in STD chickens, DFI being 3.8% 278 higher for chickens fed with AD than with CD (Table 2). 279

DFCR was highly variable between consecutive days, especially in LR chickens (Fig. 2c, 2d, 280 Supp. Table 5), while curves for DCFCR were smoothened (Fig. 3a, 3b). Thus, in LR 281 chickens DFCR was significantly better with AD for several days around the first diet change 282 (17-32 d), but better for CD for several days around the second diet change (60-68 d), 283 whereas a continuous difference was observed for DCFCR between 17 to 40 days, AD birds 284 285 being 6.8 to 13% more efficient than CD birds during this period (Supp. Table 6). Consistent with the other findings, when summarized by nutrition periods, diet effect was seen only 286 during the starter phase for DFCR, while it was seen for both the starter and grower phases for 287 DCFCR. 288

Like the LR chickens, differences of DFCR between diets in STD chickens were sporadic and limited to 5 days between 9 and 25 d (Fig. 2d, Supp. Table 5). During these 5 days, DFCR was 10.7 to 14.7 % lower for CD birds. This was confirmed by the analysis of DFCR by period (Table 2), for which a diet by period interaction was significant, due to a positive effect of the AD diet, but only during the starter phase. When considering DCFCR, diet effect was no longer significant (Fig. 3b, Table 2).

Effect on the variability. Change with age of DFI, DFCR, and DCFCR coefficients of 295 296 variation differed between traits and genotypes, although similar trends were found between diets. The general trend was an increase in the CV of the 3 traits with age in LR chickens (Fig. 297 2a, 2c, 3a) and a decrease in STD chickens (Fig. 2b, 2d, 3b). Within each genotype, the CV of 298 DFI and DFCR of LR increased with time, with a steeper slope in the starter phase than in the 299 grower and finisher phases. The CV of DCFCR of LR-CD animals increased continuously 300 301 whereas it remained stable after the first change of diet for LR-AD. In STD chickens, after a peak with high CV values during the starter phase, the CV decreased and stabilized during the 302 grower and finisher phases for DFI and DFCR. A similar profile was observed for DCFCR, 303 304 although the decrease in CV was more pronounced with AD than with CD.

Differences of variability between diets for DFI, DFCR, and DCFCR were strong in STD (Fig. 2, Fig. 3; Table 3). Alternative diet led to a decrease in the variability of those performances during the grower (DFI: -49%, DFCR: -30.4%, DCFCR: -44%) and finisher phases (DFI: -20%, DFCR: -30.4%, DCFCR: -58.4%) in STD chickens. In the case of LR chickens, the CV differed between diets during these phases for DFI and DCFCR traits. When significant, performances were less variable with the AD than with the CD diet.

#### 311 Diet effect on carcass composition and meat quality

Body composition and meat quality traits were not affected by diet in LR chickens, except for thigh yield, which was slightly higher with the AD than with the CD diet (Table 4). In STD chickens, the abdominal fat percentage was significantly lower with AD compared to CD (-14%, P<0.001). When fed with the CD diet, STD chickens had a more acidic (lower pHu) and yellower (higher b\* value) meat than those fed with AD. No diet effect was observed on the variability of the studied traits regardless of the genotype (data not shown).

# 318 Correlations between Daily Traits and Cumulative Feed Conversion Ratio or Slaughter 319 Traits (Supp. Table 7).

320 *Feed Intake*. On the whole, DFI was positively correlated with the CFCR<sub>f</sub> (Figure 4). 321 In LR chickens, the correlation was lower during the starter phase (0.32 with AD, 0.22 with CD), increased during the grower phase (0.44 with AD, 0.47 with CD), and remained stable 322 during the finisher phase (0.50 with AD, 0.61 with CD). In STD chickens, DFI and CFCR<sub>f</sub> 323 were poorly correlated during the starter phase (on average 0.23 with AD and 0.40 with CD). 324 During the grower phase, the correlation became stable and reached a higher level with CD 325 (0.62 on average) than with AD (0.21 on average). During the finisher phase, a high 326 327 correlation between DFI and CFCR<sub>f</sub> was maintained for STD chickens fed with CD diet (0.61), whereas it increased for those fed with the AD diet (0.53). 328

329 On the other hand, a moderate correlation with slaughter weight was observed for LR chickens starting at the first change of diet, stronger for those fed with AD (0.36) than with 330 331 CD (0.24). We also observed a moderate positive correlation between DFI and breast final pH 332 for these animals, particularly during the finisher phase (0.23 with CD, 0.32 with AD), whereas this correlation was low and negative in STD chickens (-0.04 with CD, -0.13 with 333 AD). In STD chickens fed AD, the correlation between DFI and pHu was strongest at the 334 beginning of the grower phase (-0.30 between 25.7 and 40 % of the age at slaughter). During 335 the same period, DFI was positively correlated with slaughter weight, as well as breast and 336 abdominal fat yields (0.50, 0.40, and 0.30, respectively), whereas these correlations became 337 weak during the finisher phase. 338

Body Weight. As expected, the correlation between daily BW and slaughter weight increased with time to reach 1 on the last day (Figure 5). Even at the youngest ages, this correlation was found to be higher than 0.50, independently of the treatment. Correlations

between body weight and other slaughter traits were weak and rather stable with age in LR 342 343 chickens. We only observed moderate positive correlations in LR-AD birds with fatness during the starter phase (0.32) and breast yield during the finisher phase (0.31). During this 344 period, a moderate, positive correlation was also found with thigh yield in LR-CD chickens 345 (0.23). In contrast, corresponding correlations varied with age or diet in STD chickens. Thus, 346 correlations with meat ultimate pH or CFCR<sub>f</sub> were stable across ages, but more pronounced 347 with the AD than with the CD diet (-0.39 and 0.01 for pHu and -0.43 and -0.27 for CFCRf, 348 respectively). While weak correlations were found between daily BW and thigh yield for both 349 diets, different profiles were found for fat yield, the correlation being stable and moderate 350 351 (0.32) for STD-CD chickens, but low for STD-AD chickens. Finally, correlations between daily BW and breast yield increased with age and reached quite significant values during the 352 finisher phase in STD chickens (0.58 with AD, 0.56 with CD). 353

Cumulative Feed Efficiency. As expected, the correlation between DCFCR and 354 CFCR<sub>f</sub> increased with age to reach 1 at slaughter (Figure 6). For LR chickens, better 355 efficiency was associated with a higher breast yield and weight at slaughter, especially with 356 CD (-0.29 and -0.47, respectively). Similar trends were observed for STD chickens during the 357 finisher phase (-0.27 and -0.36 with AD, -0.33 and -0.34 with CD). Abdominal fat percentage 358 359 and thigh yield were poorly correlated with DCFCR. Finally, a lower breast meat pH and thus more acidic meat was associated with a lower DCFCR, at least for LR-CD chickens during 360 the grower and finisher phases (0.40). This trend was not found in STD chickens. 361

362

#### 363 **DISCUSSION**

The aim of our study was to determine the capacity of adaptation of slow and fast-growing chickens to a diet containing a mixture of alternative feedstuffs, in real conditions of

production, i.e. on floor and in group. Previous studies showed that FI recorded in cages 366 differed from FI recorded on the floor. However, since many factors such as sex, diet 367 composition, and cage or litter material influenced FI, BW, and FCR, the results of these 368 studies were inconsistent (Akpobome and Fanguy, 1992; Plavnik et al., 2002; Santos et al., 369 2008; Simsek et al., 2014; Zhao et al., 2015). Automatons have already been developed to 370 record FI on the floor. However, none are capable of simultaneously measuring FI and body 371 weight throughout the whole life of animals and without limiting the expression of natural 372 behaviours due to the presence of systems of isolation of animals (Bley and Bessei, 2008; 373 Howie et al., 2009; Tu et al., 2011; Basso et al., 2014; Yan et al., 2019). Thus, only synthetic 374 375 FCR could be obtained with those automatons while ours is able to measure the kinetics of these types of traits. 376

The current study showed that differences between the two diets are moderate in terms of 377 final performances in both genotypes, indicating that chickens are able to adapt to a diet 378 composed of a mixture of alternative feedstuffs, with a higher proportion of wheat than corn 379 and a partial replacement of soybean by DDGS, rapeseed, fava bean, and sunflower meals. 380 The literature on the adaptation of chickens to a partial substitution of soybean by these 381 feedstuffs showed contrasted results both in slow- and fast-growing chickens. Depending on 382 383 the study, alternative diets led to better, similar, or worse FCR (Alagawany et al., 2017; Bosco et al., 2013; Diaz et al., 2006; Foltyn et al., 2013; Koivunen et al, 2016; Méda et al., 2015; 384 Toghyani et al., 2017). An absence of effect on FCR does not necessarily mean that there is 385 no effect on performances that contribute to FCR. For example, for the LR chickens in this 386 study as well as for the STD chickens in Diaz et al. (2006), the absence of effect of the 387 alternative diet on FCR was due to a joint increase in FI and BW rate with AD. This 388 discrepancy between studies could be due to many factors such as the animals (genotype, age, 389 390 sex) and the feedstuffs (quality, fiber percentage, and transformation process). The most

391 striking difference in the adaptability of chickens to the alternative diet was found in the 392 variability of performances. Animals fed with the alternative diet had more homogeneous 393 performances for FI and daily and cumulative FCR, especially in STD chickens.

Another interest of the daily data is that it highlighted the importance of transition periods 394 around diet changes. Modifications of performances around the time of the diet change could 395 indicate a difficulty in adapting to the new diet if it appears after the transition or a necessity 396 to change the diet earlier if it appears before the transition. These modifications are genotype 397 and diet dependent and could be linked to several factors. For example, some diets has been 398 shown to modify the development of digestive tract and thus its capacity of absorption 399 400 (Nassiri Moghaddam et al., 2012). A difference of palatability between successive diets can be a cause of variations occurring after transitions. The drop we observed in weight gain 401 despite the continuous increase in FI before the second diet change for the LR-AD chickens 402 403 can suggest that the animals' needs are not fulfilled anymore and that the diet change should have been done 3 to 4 days earlier, whereas this is not the case with the classic diet or with the 404 405 STD chickens. Similarly, the strong increase in the coefficient of variation of FI in STD chickens before the first diet change may indicate that this diet change occurs too late for 406 some of the birds. This daily information could also help us to identify animals that are 407 resilient to disturbances in their environment, especially around times of dietary transitions. 408

Finally, the correlation profiles between daily measurements and phenotypes measured at slaughter are useful to understand early indicators of final phenotypes. These indicators differ between genotypes and diets, which also highlights the fact that final phenotype construction differs between genotypes and diets. For example, DFI is a good indicator of final FCR in STD chickens fed with CD, as the correlation between both traits is high as early as the first diet change. In contrast, when fed with AD, the correlation between both traits increased later, after the second diet change. The correlations between BW, AFY, BMY, and breast meat pHu

in STD chickens also show that animals do not respond to CD and AD in the same way. For 416 417 instance, although increased BW at early ages appears to be an indicator of increased breast meat yield at slaughter for both diets, it also seems to be associated with higher muscle 418 glycogen reserves which are the cause of lower ultimate pH (Le Bihan-Duval et al., 2008) for 419 birds fed the AD, and of higher abdominal fatness for birds fed the CD. This is maybe why 420 the correlation between BW at an early age and CFCR<sub>f</sub> seems a little bit lower with CD than 421 422 with AD, the energy cost of glycogen deposition in breast muscle being lower than the energy cost of abdominal fat deposition. In the current study, we also found indications showing that 423 better FCR at early ages could be a predictor of higher breast development at slaughter in LR 424 425 chickens, and could be of interest to limit the production costs of this alternative production and to satisfy the needs of the growing market of cuts and further processed products. 426

To conclude, both genotypes showed a good ability to adapt to alternative diets. Taking into 427 account the costs of feedstuffs and mean feed intake, using these alternative diets would 428 increase feed cost by 1.5% for LR chicken and 3.4% for the STD chicken, close to the 0.5-4% 429 of increasing already found in literature (Nguyen et al., 2011). This represents an increase of 430 respectively 0.9% and 2% of the total production costs (Chenut, 2016). However, it has been 431 shown that replacing soybean by local feedstuffs can decrease greenhouse gas emission up to 432 41% depending of the percentage of replacement and the genotype (Méda et al., 2015). This 433 element is important to evaluate the environmental impact of both diets which has to be taken 434 into account in the perspective of making poultry meat production more sustainable. 435

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Table 1. Composition and age of distribution of classical (CD) and alternative (AD) diets for standard (STD) and Label Rouge (LR) genotypes.

	STD					LR						
		CD			AD			CD			AD	
Ingredient (%)	1-7 d	8-22 d	23-35 d	1-7 d	8-22 d	23-35 d	1-28 d	29-63 d	64-82 d	1-28 d	29-63 d	64-82 d
Corn	30.650	35.970	39.800	20.420	18.890	23.500	29.620	42.920	46.620	18.250		16.950
Wheat	30.100	30.100	30.100	30.100	30.100	30.100	38.550	30.100	30.100	40.100	57.950	45.100
Fava bean					12.000	13.000				10.000	13.000	12.000
Soybean meal	32.860	28.520	25.150	24.220	11.610	7.130	28.080	23.160	19.840	18.540	6.730	5.200
Rapeseed meal				5.000	5.000	8.000				5.000	5.000	5.000
Wheat DDGS				3.000	5.000	5.000				3.000	5.000	
High fiber sunflower meal				8.120	7.730	5.190					5.020	8.000
Soybean oil	2.210	1.900	1.990	5.000	5.000	5.000		0.360	0.570	1.420	3.820	3.800
Corn gluten												1.100
Calcium carbonate	0.710	0.169	0.002	0.655	0.142		0.600	0.274	0.300	0.590	0.390	0.350
Bicalcic phosphate	2.160	1.850	1.540	2.050	1.730	1.400	1.970	1.870	1.560	1.880	1.540	1.350
Salt	0.236	0.207	0.211	0.192	0.150	0.158	0.270	0.246	0.280	0.254	0.180	0.230
Vitamins and minerals	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Sodium carbonate	0.135	0.173	0.175	0.196	0.262	0.250	0.100	0.129	0.081	0.114	0.227	0.160
DL-Methionine	0.269	0.275	0.231	0.234	0.285	0.234	0.204	0.211	0.114	0.207	0.230	0.114
HCL Lysine	0.176	0.264	0.250	0.287	0.414	0.392	0.154	0.243	0.125	0.183	0.373	0.214
Threonine	0.076	0.111	0.094	0.088	0.157	0.135	0.052	0.087	0.010	0.062	0.140	0.032
Valine	0.021	0.061	0.041	0.038	0.130	0.106						
Tryptophane						0.005						
Calculated composition												
AMEn, kcal/kg	2850	2900	2950	2850	2900	2950	2750	2850	2900	2750	2850	2890
CP, g/kg	215.0	200.4	187.1	215.0	194.3	181.3	200.0	179.8	165.0	200.0	179.0	164.9
Lys, g/kg	11.200	10.900	10.000	11.200	10.900	10.000	10.000	9.500	7.800	10.000	9.500	7.810
Met + Cys, $g/kg$	8.400	8.170	7.500	8.400	8.170	7.500	7.500	7.200	6.000	7.500	7.200	6.000
Trp, g/kg	2.280	2.060	1.890	2.280	1.840	1.700	2.100	1.790	1.620	1.990	1.730	1.490

Table 2. Diet and period effects on body weight (BW), average daily gain (ADG), feed intake (DFI), feed efficiency (DFCR) and cumulated feed efficiency

### (DCFCR).

			Label Rouge chickens						Sta	ndard chick	cens	
			BW (g)	ADG (g.d <sup>-1</sup> )	DFI (g.d <sup>-1</sup> )	DFCR	DCFCR	BW (g)	ADG (g.d <sup>-1</sup> )	DFI (g.d <sup>-1</sup> )	DFCR	DCFCR
	Diet <sup>1</sup>	AD	$1472^{a}$	31.7 <sup>a</sup>	98.3	3.09 <sup>b</sup>	1.95 <sup>b</sup>	724	47.8	93.6b	1.79 <sup>b</sup>	1.44
	Diet	CD	1431 <sup>b</sup>	31 <sup>b</sup>	98.1	3.20 <sup>a</sup>	2.05 <sup>a</sup>	727	46.8	90.2a	1.86 <sup>a</sup>	1.46
	Period <sup>2</sup>	S	370 <sup>c</sup>	21.3 <sup>c</sup>	46.5 <sup>c</sup>	2.32 <sup>c</sup>	1.70 <sup>c</sup>	121 <sup>c</sup>	17.3 <sup>c</sup>	27.9 <sup>c</sup>	1.67 <sup>c</sup>	1.25 <sup>b</sup>
		G	1366 <sup>b</sup>	37.9 <sup>b</sup>	104.2 <sup>b</sup>	2.82 <sup>b</sup>	1.94 <sup>b</sup>	498 <sup>b</sup>	42.8 <sup>b</sup>	77.7 <sup>b</sup>	1.85 <sup>b</sup>	1.53 <sup>a</sup>
LS		F	2620 <sup>a</sup>	35.0 <sup>a</sup>	143.8 <sup>a</sup>	4.28 <sup>a</sup>	2.36 <sup>a</sup>	1558 <sup>a</sup>	81.9 <sup>a</sup>	170.1 <sup>a</sup>	1.96 <sup>a</sup>	1.57 <sup>a</sup>
Means <sup>3</sup>		AD×S	381	22.1 <sup>d</sup>	45.9	2.20	1.64 <sup>e</sup>	117	17.5	28.1	1.54 <sup>d</sup>	1.26
		AD×G	1398	38.6 <sup>a</sup>	105.8	2.80	1.86 <sup>c</sup>	493	43.1	76.6	1.86 <sup>bc</sup>	1.51
	Diet×Period	AD×F	2639	34.5 <sup>c</sup>	143.1	4.26	2.35 <sup>a</sup>	1562	82.9	174.2	$1.97^{a}$	1.55
	Diet×Feilou	CD×S	359	20.5 <sup>d</sup>	47.1	2.44	1.77 <sup>d</sup>	125	17.1	27.7	1.79 <sup>c</sup>	1.24
		CD×G	1334	37.1 <sup>b</sup>	102.6	2.84	$2.02^{b}$	503	42.4	76.8	1.85 <sup>c</sup>	1.56
		CD×F	2601	35.5°	144.5	4.30	2.38 <sup>a</sup>	1554	80.9	166.0	1.95 <sup>ab</sup>	1.57
	Diet		0.003	0.020	0.870	0.010	0.001	0.811	0.090	0.050	0.030	0.506
P-value	Period	b	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	Diet×Per	riod	0.277	0.001	0.107	0.100	0.001	0.842	0.432	0.070	0.001	0.523

<sup>1</sup> AD: alternative diet; CD: control diet

 $^{2}$  S: starter diet (d1 to d7 for STD, d1 to d28 for LR); G: grower diet (d8 to d22 for STD, d29 to d63 for LR); F: finisher diet (d23 to d35 for STD, d69 to d82 for LR)

<sup>3</sup> within effect, trait and genotype, LS means values with different superscripts are significantly different (P<0.05)

		Label Rouge chickens							Standard chickens					
			BW (%)	ADG (%)	DFI (%)	DFCR (%)	DCFCR (%)	BW (%)	ADG (%)	DFI (%)	DFCR (%)	DCFCR (%)		
	Diet <sup>1</sup>	AD	$8.7^{\mathrm{b}}$	20.3	21.6 <sup>a</sup>	28.0 <sup>a</sup>	18.0 <sup>b</sup>	10.8 <sup>a</sup>	13.0	20.5 <sup>b</sup>	23.9 <sup>b</sup>	33.1 <sup>b</sup>		
	Diet	CD	9.5 <sup>a</sup>	22.6	27.4 <sup>b</sup>	31.0 <sup>b</sup>	24.0 <sup>a</sup>	8.5 <sup>b</sup>	12.9	25.0 <sup>a</sup>	34.3 <sup>a</sup>	43.0 <sup>a</sup>		
	Period <sup>2</sup>	S	8.8 <sup>b</sup>	14.1 <sup>c</sup>	15.9 <sup>a</sup>	19.3 <sup>a</sup>	16.1 <sup>c</sup>	<b>8</b> .5 <sup>b</sup>	13.4	26.9 <sup>a</sup>	42.3 <sup>a</sup>	52.4 <sup>a</sup>		
		G	9.1 <sup>ab</sup>	20.5 <sup>b</sup>	25.8 <sup>b</sup>	$32.0^{b}$	$22.4^{b}$	$10.0^{a}$	13.0	22.0 <sup>b</sup>	23.4 <sup>b</sup>	36.1 <sup>b</sup>		
LS		F	9.3 <sup>a</sup>	29.8 <sup>a</sup>	31.8 <sup>c</sup>	37.0 <sup>c</sup>	34.5 <sup>a</sup>	10.4 <sup>a</sup>	12.5	19.4 <sup>c</sup>	21.5 <sup>c</sup>	25.6 <sup>c</sup>		
LS Means <sup>3</sup>		AD×S	8.7 <sup>c</sup>	11.1 <sup>d</sup>	16.1 <sup>d</sup>	16.2 <sup>c</sup>	15.2 <sup>e</sup>	9.8	13.6	26.6 <sup>a</sup>	37.6	58.4 <sup>a</sup>		
		AD×G	8.4 <sup>c</sup>	21.7 <sup>bc</sup>	23.9 <sup>c</sup>	32.4 <sup>b</sup>	19.9 <sup>c</sup>	11.5	13.5	17.7 <sup>c</sup>	16.7	26.1 <sup>c</sup>		
	Diet×Period	AD×F	9.0 <sup>bc</sup>	28.1 <sup>ab</sup>	24.9 <sup>bc</sup>	35.2 <sup>ab</sup>	18.8 <sup>cd</sup>	11.2	11.9	17.2 <sup>bc</sup>	17.2	14.7 <sup>d</sup>		
	2100 1 01100	CD×S	8.9 <sup>c</sup>	17.1 <sup>cd</sup>	15.7 <sup>d</sup>	22.5 <sup>°</sup>	17.0 <sup>de</sup>	7.3	13.2	$27.2^{a}$	47.0	46.3 <sup>ab</sup>		
		CD×G	<b>9.8</b> <sup>a</sup>	19.3 <sup>c</sup>	27.7 <sup>b</sup>	31.4 <sup>b</sup>	$24.8^{b}$	8.6	12.3	26.4 <sup>a</sup>	30.0	46.1 <sup>a</sup>		
		CD×F	9.7 <sup>ab</sup>	31.6 <sup>a</sup>	38.7 <sup>a</sup>	38.8 <sup>a</sup>	30.1 <sup>a</sup>	9.6	13.3	21.5 <sup>ab</sup>	25.8	36.4 <sup>b</sup>		
	Diet		0.001	0.130	0.001	0.020	0.001	0.001	0.940	0.001	0.001	0.001		
P-value	Period	l	0.010	0.001	0.001	0.001	0.001	0.001	0.615	0.001	0.001	0.001		
	Diet×Per	iod	0.002	0.046	0.001	0.036	0.001	0.192	0.105	0.020	0.276	0.001		

**Table 3.** Diet and period effects on the coefficient of variation of body weight (BW), average daily gain (ADG), feed intake (DFI), feed efficiency (DFCR) and cumulated feed efficiency (DCFCR) for each chicken genotype.

<sup>1</sup> AD: alternative diet; CD: control diet

<sup>2</sup> S: starter diet (d1 to d7 for STD, d1 to d28 for LR); G: grower diet (d8 to d22 for STD, d29 to d63 for LR); F: finisher diet (d23 to d35 for STD, d69 to d82 for LR)

<sup>3</sup> within effect, trait and genotype, LS means values with different superscripts are significantly different (P<0.05)

- 1 **Table 4**. Body composition and meat characteristics of label rouge (LR) and Cobb500 (STD)
- 2 genotypes fed with either the alternative diet or the classical diet.
- 3

		LS N	<i>P</i> -value	
Trait <sup>1</sup>	Genotype	D	of diet effect	
		AD	CD	
Slaughter	LR	3010	2951	0.371
weight (g)	STD	2334	2355	0.720
AFY (%)	LR	3.53	3.95	0.080
	STD	1.57	1.83	0.001
BMY (%)	LR	14.56	14.40	0.550
	STD	20.44	20.40	0.970
TY (%)	LR	25.64	25.16	0.030
	STD	22.58	22.94	0.100
L*	LR	48.76	49.14	0.520
	STD	47.99	47.38	0.250
a*	LR	-1.06	-1.09	0.860
	STD	-0.51	-0.72	0.100
b*	LR	9.82	9.48	0.230
	STD	8.02	8.89	0.001
pHu	LR	5.74	5.72	0.350
	STD	5.89	5.79	0.001

4

6 luminance, a\*: breast meat redness, b\*: breast meat yellowness, pHu: breast meat pH 24 h

7 after slaughter

8 <sup>2</sup> AD: alternative diet; CD: control diet

<sup>5 &</sup>lt;sup>1</sup> AFY: abdominal fat yield, BMY: breast muscle yield, TY: thigh yield,  $L^*$ : breast meat

**Figure 1.** Kinetics of the mean (solid line) and of the coefficient of variation (dotted line) for BW (1a for LR; 1b for STD) and ADG (1c for LR; 1d for STD) for chickens fed with classical diet (in red) or alternative diet (in blue). Black vertical lines are indicating diet changes. Green horizontal lines are indicating the periods of significance of the diet effect.

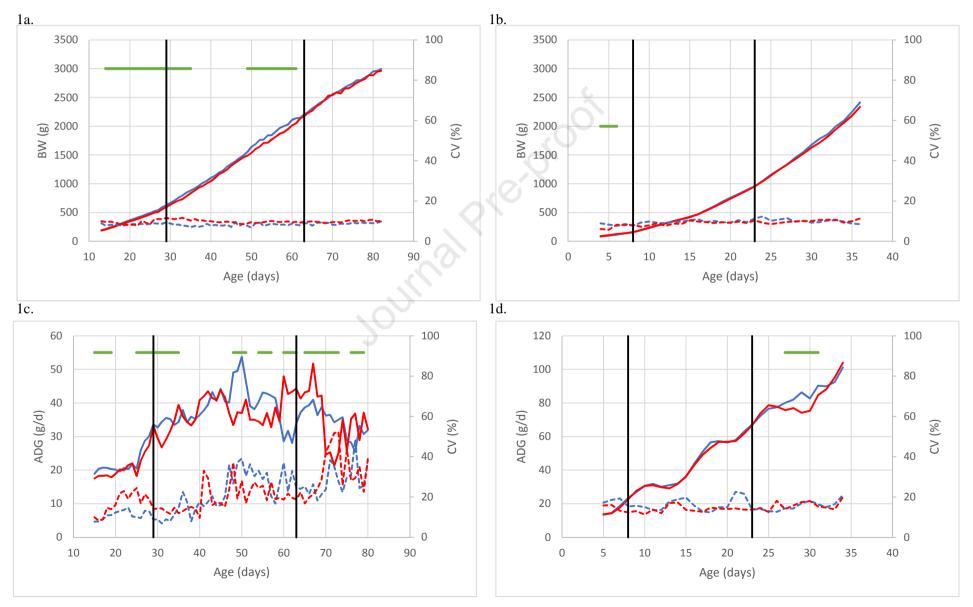


Figure 2. Kinetics of the mean (solid line) and of the coefficient of variation (dotted line) for DFI (2a for LR; 2b for STD) and DFCR (2c for LR; 2d for STD) for chickens fed with classical diet (in red) or alternative diet (in blue). Black vertical lines are indicating diet changes. Green horizontal lines are indicating the periods of significance of the diet effect.

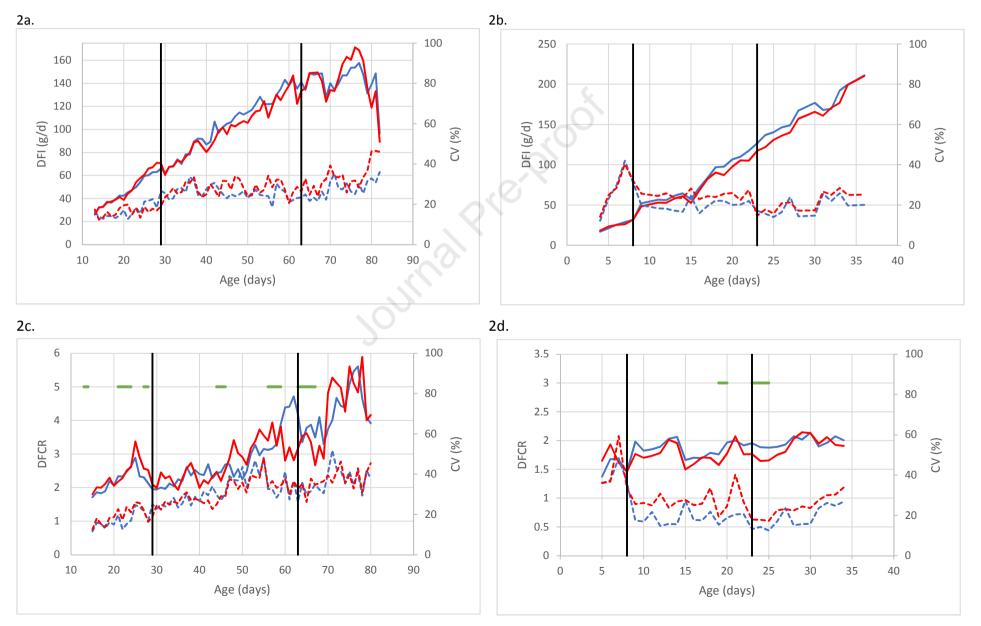
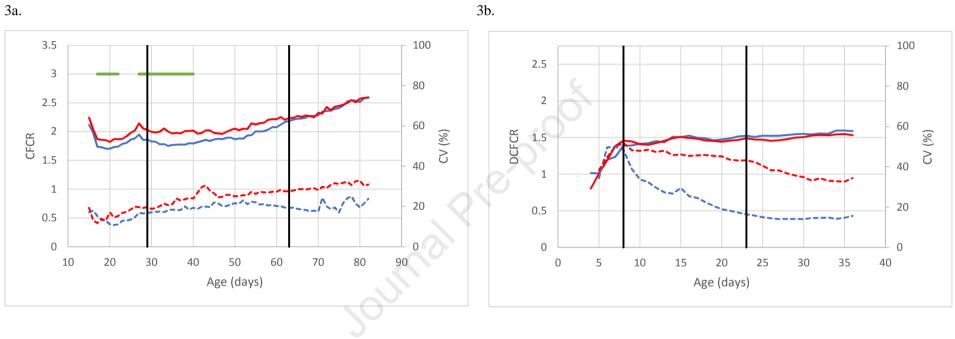
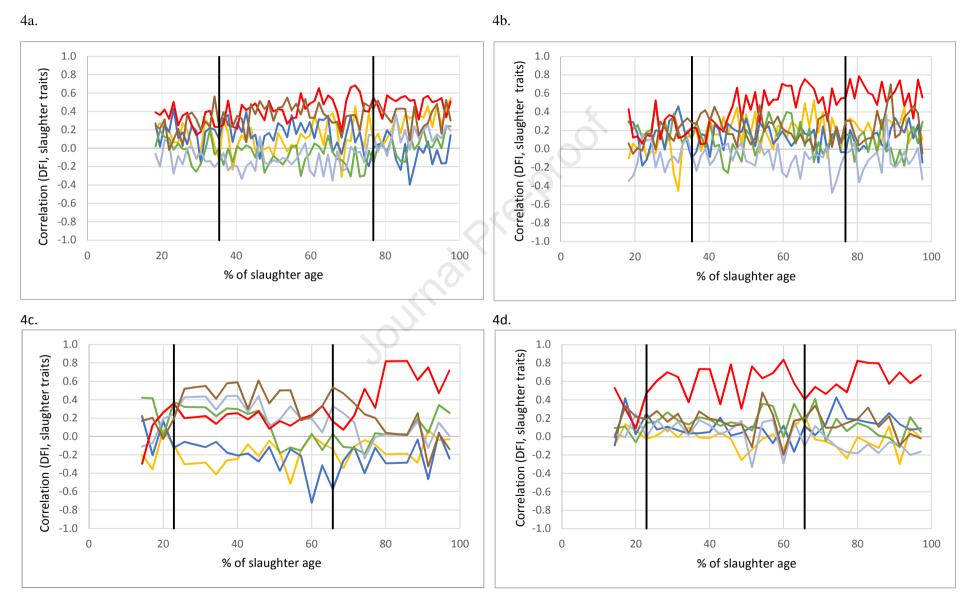


Figure 3. Kinetics of the mean (solid line) and of the coefficient of variation (dotted line) for DCFCR (1a for LR; 1b for STD) for chickens fed with classical diet (in red) or alternative diet (in blue). Black vertical lines are indicating diet changes. Green horizontal lines are indicating the periods of significance of the diet effect.

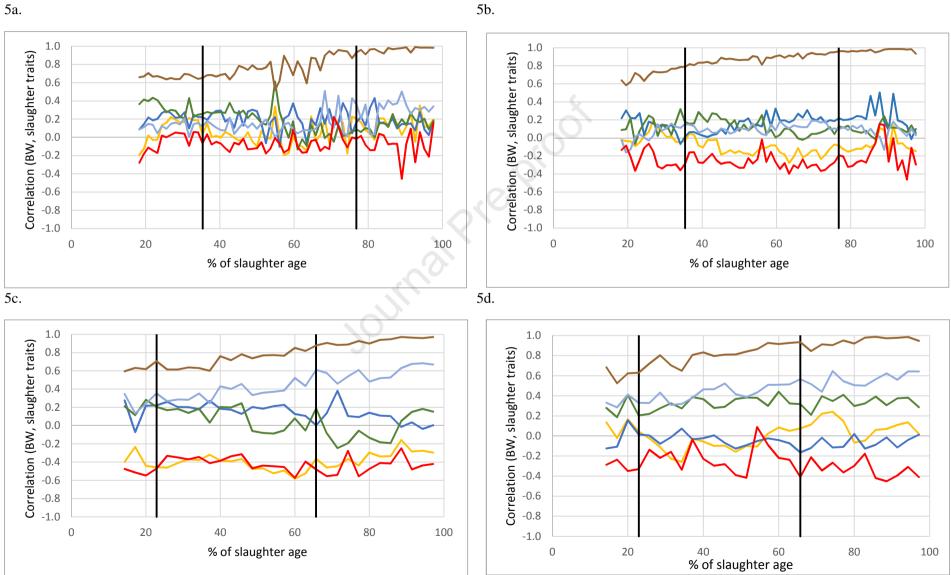


**Figure 4.** Profiles of correlations for LR (a: AD, b: CD) and STD (c: AD, d: CD) chickens between DFI and traits measured at slaughter (pHu in yellow, thigh yield in dark blue, AFP in green, BMY in light blue, CFCRf in red, BW at slaughter in brown). Black lines indicate diet changes.



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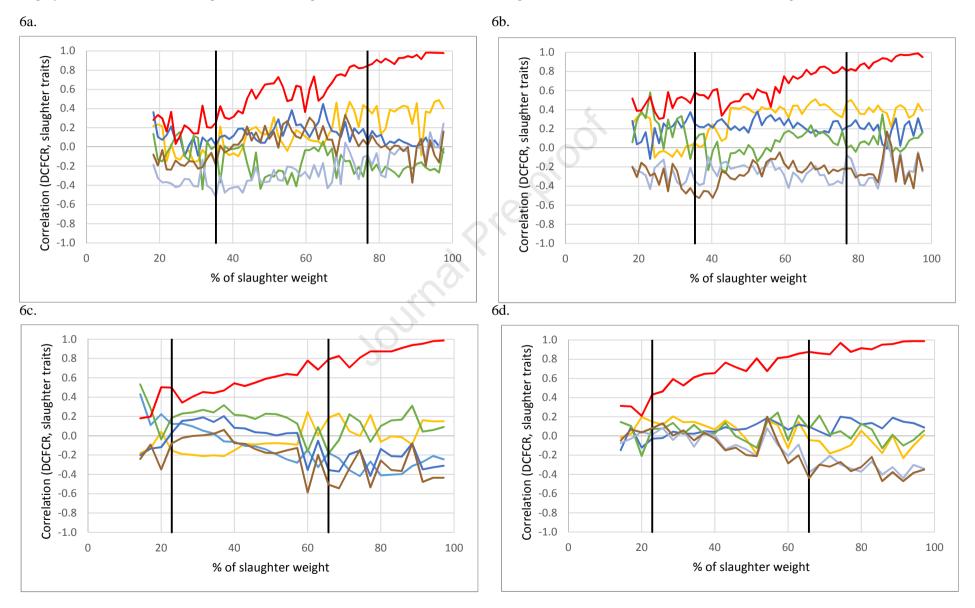
Figure 5. Profiles of correlations for LR (a: AD, b: CD) and STD (c: AD, d: CD) chickens between BW and traits measured at slaughter (pHu in yellow, thigh yield in dark blue, AFP in green, BMY in light blue, CFCRf in red, BW at slaughter in brown). Black lines indicate diet changes.



5b.

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**Figure 6.** Profiles of correlations for LR (a: AD, b: CD) and STD (c: AD, d: CD) chickens between DCFCR and traits measured at slaughter (pHu in yellow, thigh yield in dark blue, AFP in green, BMY in light blue, CFCRf in red, BW at slaughter in brown). Black lines indicate diet changes.



The authors declare they have no conflict of interest.

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