Combination of 9 Assays for Calculation of Relationships between Wheat AMEn Values in Broilers and Wheat Physicochemical Parameters.

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Summary
Two experiments (1 and 2) were conducted to complete the calculations of relationships between AMEn value of wheat samples and their physical or chemical parameters conducted in two previous experiments (Carré et al., 2002, 2005). Experiments 1 and 2 were each divided into three identical assays conducted with 3 week interval. Twenty and 21 wheat samples were tested in experiments 1 and 2, respectively, with 15 individual replicates (Ross chickens maintained in individual cages, 5 replicates per wheat and assay) per wheat samples. Wheat samples were introduced at a level of 56% in pelleted diets. Birds were fed the wheat experimental diets from 16d. AMEn value of diets was determined in each bird from 21d to 23d. AMEn values assigned to wheat samples were calculated for each bird according to the AMEn additivity principle, using a constant AMEn value for each of the other ingredients of diets. Data from these two experiments were combined to previous ones (Carré et al., 2002, 2005). So, calculations combined 9 assays including 77 wheat samples and a total of 893 individual chicken data. The mean wheat AMEn value was 13,396 (J/g DM). Effects of assays, wheat Water-Insoluble Cell Wall (WICW) content (% DM), wheat hardness (0 to 100) and in vitro viscosity of wheat aqueous extract (Real Applied Viscosity, RAV, ml/gDM) combined together were all significant (P = 0.0001, except for RAV : P = 0.0003) on individual wheat AMEn values, with negative coefficients of -144.5, -4.69 and -139.8 for WICW, hardness and RAV, respectively.

Keywords: broiler, wheat, viscosity, hardness, NIR

Introduction
The nutritional value of wheat for broilers has been shown to vary in a wide range (Carré et al., 2002). So, feed manufacturers often limit the wheat level in mixed feed. However, such limitation reduces the possibility of optimization for the feed formulation. Choosing wheat cultivars with high nutritional value could be a good solution for reducing the risks induced by wheat feeding. Several studies have been conducted in the past in our laboratory (Carré et al., 2002, 2005) for identifying the parameters of wheat cultivars that affect the nutritional value of wheat for broilers. In vitro viscosity and hardness values were shown to be negative factors for the AMEn value of wheat diets (Carré et al., 2002, 2005).
However, the significance levels of effects were rather low, which resulted in low precision for the coefficients assigned to wheat parameters (Carré et al., 2002, 2005). The main reason of such low precisions was the high variability of bird responses. We know now that this variability is associated with the genetic variability of broilers (Mignon-Grasteau et al., 2004). Owing to this high variability, studies on wheat nutritional value in broilers should be conducted with a great number of bird replicates and wheat samples. The current publication reports calculations combining the results of four experiments, with two being already published (Carré et al., 2002, 2005) and two others being presented in the current publication. So, these calculations combined about 900 individual bird digestibility measurements and 77 wheat samples.

Materials and methods

**Animals and diets.** Two experiments (1 and 2) were conducted in 2007 and 2009, respectively, for the determination of AMEn and digestibilities in male broiler chickens (Ross PM3) fed on various wheat experimental diets differing in the origin of wheat samples. Twenty and 21 different wheat samples were tested in experiments 1 and 2, using 300 and 315 birds, respectively. Each experiment was divided into 3 identical assays conducted with 3 weeks interval. In each assay, each experimental diet was given to 5 chickens. So, in each experiment, each wheat experimental diet was given to 15 chickens, with 5 chickens per assay. From 0d to 9d, birds were reared altogether on litter floor with a standard commercial starter diet. At 9d, birds were assigned at random to individual cages and fed a standard wheat pelleted diet up to 16d. At 16d, birds were assigned at random to wheat experimental diets and ad libitum fed up to 23d. Diets contained 56% wheat, 30.8% soybean meal, 6% maize gluten meal and 3% rapeseed oil. All diets were pelleted before feeding. A balance experiment was conducted for 3 days from 20d, with total collection of excreta and no starvation periods, as described by Péron et al. (2005). In experiment 1, the control of feed intake by measuring acid-insoluble ashes (AIA) in feed and excreta revealed no significant difference with the feed intake measured by feeder weighing at the end and beginning of balance period. This was already observed in the past (Péron et al., 2005). So, in experiment 2, Celite was not incorporated in feed, and all feed intake data were derived from feeder weighing for both experiments.

**Analyses.** AIA in feed and excreta, and gross energy, nitrogen, starch and lipid in feed were measured as previously described by Carré et al. (2002) and Péron et al. (2005). Uric acid and the latter components were measured in freeze-dried excreta with the assistance of NIR spectroscopy, as described by Bastianelli et al. (2010), using the chemical methods described by Péron et al. (2005) for NIR calibration. Protein nitrogen of excreta was calculated as the difference between total and uric acid nitrogen contents. Water-insoluble cell-walls (WICW) contents, in vitro viscosity values as Potential or Real Applied Viscosity (PAV or RAV), and hardness were measured for each wheat samples, as described by Carré et al. (2002), using NIR for wheat hardness determination.

**Calculations.** For each bird, the AMEn value assigned to the wheat fraction of diets was calculated from the AMEn value of diet, using the additivity principle and constant AMEn values for the remaining part of diets, using AMEn values of 33,982, 10,532 and 14,266 J/g DM for rapeseed oil, soybean meal 48 and gluten meal 60, respectively. These calculations were applied on data from experiments 1 and 2, and also from Carré et al. (2002, 2005).

**Statistical Analyses.** Statistical analyses were performed with the SuperAnova software (Abacus Concepts, Inc), applying the type III technique for the calculation of sums of squares.
ANOVA calculations, multiple regression lines, and multiple regression lines combining the effect of assays were performed. Data from 9 assays were combined together, namely 6 assays from experiments 1 and 2, and 3 assays from Carré et al. (2002, 2005). Experimental values were considered outliers when residues were 3 fold higher than the value of residual standard deviation.

**Results and discussion**

Ranges of variations for wheat physicochemical parameters and nutritional data observed in experiments 1 and 2 are shown on Table 1. Better nutritional values were observed in Experiment 2, except for lipid digestibility. This was not due to differences in wheat parameters, since multiple regression analyses combining both experiments and calculating Table 1. Means and range of physicochemical parameters and nutritional values of wheat samples and diets

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1 (20 samples)</th>
<th>Experiment 2 (21 samples)</th>
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<tbody>
<tr>
<td>Wheat samples</td>
<td></td>
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<tr>
<td>Crude Protein (% DM)</td>
<td>12.42 (9.69 - 14.79)</td>
<td>13.85 (10.29 - 16.59)</td>
</tr>
<tr>
<td>WICW(^1) (% DM)</td>
<td>11.06 (9.36 - 13.79)</td>
<td>11.18 (9.72 - 12.46)</td>
</tr>
<tr>
<td>PAV(^2) (ml/g DM)</td>
<td>3.74 (2.81 - 5.41)</td>
<td>3.95 (2.01 - 6.26)</td>
</tr>
<tr>
<td>RAV(^2) (ml/g DM)</td>
<td>2.46 (1.36 - 3.46)</td>
<td>2.30 (1.25 - 3.78)</td>
</tr>
<tr>
<td>Hardness(^3)</td>
<td>52.6 (7 - 93)</td>
<td>28.7 (-2 - 66)</td>
</tr>
<tr>
<td>AMEn (J/g DM)</td>
<td>13,094 (12,469 - 13,550)</td>
<td>13,948 (13,378 - 14,366)</td>
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<tr>
<td>Diets</td>
<td></td>
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<tr>
<td>AMEn (J/g DM)</td>
<td>12,510 (12,168 - 12,760)</td>
<td>13,056 (12,738 - 13,288)</td>
</tr>
<tr>
<td>Total Apparent Starch Digestibility (%)</td>
<td>93.39 (90.14 - 96.80)</td>
<td>96.18 (92.80 - 98.00)</td>
</tr>
<tr>
<td>Total Apparent Protein Digestibility (%)</td>
<td>80.45 (79.17 - 81.63)</td>
<td>86.28 (84.49 - 87.72)</td>
</tr>
<tr>
<td>Total Apparent Lipid Digestibility (%)</td>
<td>81.72 (78.94 - 84.62)</td>
<td>82.51 (79.42 - 85.14)</td>
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\(^1\)Water-Insoluble Cell-Wall
\(^2\)PAV: Potential Applied Viscosity; RAV: Real Applied Viscosity.
\(^3\)Hardness value determined by NIR spectroscopy.
Figure 1. Individual measured wheat AMEn$^1$ (J/g DM), in 3 wk broilers

\[
\text{Individual predicted wheat AMEn (J/g DM) = 15,520} - 144.5 \text{WICW (\% DM)} - 139.8 \text{RAV (ml/g DM)} - 4.69 \text{Hardness} \\
R^2 = 0.072; \text{RSD = 720; n = 858 chickens (without outliers); } P = 0.0001
\]

WICW: Water-Insoluble Cell-Wall content of wheat
RAV: Real Applied Viscosity value of wheat
Outlier: |measured - predicted value| > 3 RSD

$^1$ Measured wheat AMEn values were corrected for the assay effects observed in the analysis testing assay, WICW, RAV and Hardness.

Pooled data from experiments 1 and 2, and from Carré et al. (2002, 2005).

nutritional data as functions of wheat parameters gave rise to a strong experiment effect. Birds from experiment 2 showed much less sensitivity to variations in wheat quality, compared to birds from experiment 1. Despite a common commercial origin, it is possible that broilers differed in their genetic quality between experiments, since 2 years separated experiments. It is noteworthy that, among all experiments conducted at INRA over the last 14 years, the experiment conducted in 2009 produced the highest wheat nutritional values, with 7 and 9% higher AMEn and protein digestibility values compared to previous experiments. However, anatomic characteristics of birds were not examined. So, no information could confirm a difference in genetic features for birds from experiment 2.

Individual data from experiments 1 and 2 were combined to those obtained in two previous experiments (Carré et al., 2002, 2005). So, 893 individual data and 77 different wheat samples could be examined together (Figure 1). The mean wheat AMEn value from these data, excluding 35 outliers (Figure 1), was 13,396 (J/g DM). The assay effects were deduced from the multiple regression line calculation including the assay effect. Then, these assay effects were used to correct individual nutritional data for the assay effects. These assay effects were very similar to those deduced from a variance analysis testing both wheat (nested in assay effect) and assay effects.

Owing to the great number of birds, it was possible to perform a calculation combining the three main wheat parameters that were identified in previous studies as being the major factors associated with wheat AMEn variation (Carré et al., 2002, 2005), namely wheat WICW, RAV and hardness. This was also facilitated by rather low associations between these independent variables, since correlation r values were +0.33, -0.26 and +0.02 for the WICW-
RAV, WICW-hardness and RAV-hardness associations, respectively. The proportion of outliers defined according to this multiple regression analysis was rather low (3.9% of total birds, Figure 1).

The relationship between wheat AMEn values and these parameters is shown on Figure 1. Statistical significances of coefficients assigned to WICW, RAV and hardness were all very high ($P=0.0001$; 0.0003; 0.0001, respectively). It is noteworthy that the WICW coefficient (144.5) was very close to its theoretical coefficient of 150.5 obtained by using the principle that WICW acts as a diluter of wheat AMEn values. So, according to this equation and to physicochemical variations between wheat cultivars, genetic selection of wheat may result in about 8% variation in their AMEn value for broilers.

References


