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Blood profile and productive performance after partial substitution of maize grain with ancient wheat lines by-products in organic laying hens' diet

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ABSTRACT

The aim of this research was to evaluate the effects of the partial substitution of maize grain with local ancient wheats by-products in an organic laying hens farm on animal performance and blood profile, as well as on eggs quality. A total of 80 Hy-Line W-36 Single Comb White Leghorn, 18 weeks old hens were homogenously divided into two groups. The two groups (40 laying hens each, 8 replicates, 5 hens/replicate) were fed two isoprotein and isoenergetic diets: the Control group (C) was fed a standard, organic maize-soybean meal diet whereas in the Ancient Grains group (AG) part of the maize grain was replaced by a mix of ancient grains middling, consisting in 50:50 of *Triticum aestivum* var. *spelta* (spelt) and *Triticum durum dicoccum* L. (emmer wheat). The birds fed the maize based-diet (C) showed a higher ($P < 0.01$) egg weight and feed intake than the Ancient Grains (AG) group. By contrary, the feed conversion ratio was more favourable ($P < 0.05$) in hens fed the Ancient Grains diet. The eggs produced by the hens fed the Control diet showed higher length and width than the other group ($P < 0.01$). The shell thickness and strength were also higher in the Control group ($P < 0.01$ and $P < 0.05$, respectively). The experimental dietary treatment positively affected some blood parameters. The Control group showed higher levels of cholesterol and triglycerides ($P < 0.05$) as well as of alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) ($P < 0.01$). In addition, butyrate, which may have great implications for the regulation of the immune response, resulted significantly higher in the caecal content of hens from the Ancient Grains group. Overall, the Ancient Grains diet seemed to be able to guarantee the production performances with positive effects on the animal health.

1. Introduction

Ancient wheats are primitive grains not subjected to any modern breeding or selection, thus maintaining the characters of the wild ancestors, such as large individual variability, ear height, brittle rachis, and low harvest index (Giambanelli et al., 2013). Generally, the ancient wheat refers to emmer, einkorn, Khorasan wheat (Oriental wheat) and spelt. The increasing interest and consumption of ancient wheats in human nutrition is related to several points. First of all, they are rich in micronutrients that can apport several benefits for human health (Arzani, 2011; Randall et al., 2012; Chandi et al., 2015), reducing the risk of chronic diseases such as cardiovascular diseases, diabetes, obesity and certain cancers (Boukid et al., 2018). Some studies suggested that the consumption of ancient grains can ameliorate glucose and lipid metabolism as well as the levels of pro-inflammatory and anti-oxidant parameters (Whittaker et al., 2017). Also, they can reduce the severity of gastro-intestinal and extra-intestinal symptoms in patients affected by the irritable bowel syndrome (Boukid et al., 2018). On the other hand, recent reviews (Shewry, 2018; Dinu et al., 2018) stated that not enough studies are available on this last topic, thus, many aspects, including a wider range of genotypes of ancient wheats, are necessary.

Another attractive aspect of the ancient wheats is their environmental-friendly production, being generally cultivated in organic or traditional low-input farming, they address the current concerns for environmental sustainability (Dinu et al., 2018; Fardet, 2014). In addition, old genotypes of grains, even though much less productive than the modern ones, are perfectly suitable for marginal areas and for low-input and high-stress conditions (Ceccarelli and Grando, 1996).

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Consequently, they could represent a solution for local communities where the commonly grown varieties are not suitable (Migliorini et al., 2016).

In human nutrition, the ancient wheats are generally used as whole grains, in order to maximize the beneficial effects of their nutrients, but very often they are also used to produce pasta, bread and other products (Benincasa et al., 2015). Importantly, when these wheats are grinded to obtain flour, often in small mills, the residual by-products (middlings, brans) could be a potential source of nutrients also for animals, as an alternative to the common grains, with possible benefits on health and production. Indeed, poultry are the best candidates for feeding ancient grains by-products snice their diets are commonly based on corn and soybean. In particular when raised under alternative farm conditions, due to the low number of fared animals and the use of slow-growing strains. The most attractive farming condition for laying hens is the organic free-range system, in which birds have a high amount of available external space with positive effects on animal welfare and eggs quality (Pettersson et al., 2016).

The aim of this research was to evaluate the partial substitution of maize with ancient wheats (spelt and emmer wheat) by-products in an organic laying hens farm by detecting the effects of their inclusion in the diet on animals performance and blood profile as well as on the physical quality of eggs. To this purpose, the trial was designed in order to respect the features of the organic system. The two ancient wheats were chosen according to the easiest availability from local mills.

2. Materials and methods

2.1. Animals

All the animals were treated according to the principles of the animal welfare stated by the Directive 63/2010/EEC regarding the protection of the animals used for experimental and other scientific purposes. The experimental procedures were approved by the Ethical Animal Care and Use Committee of the Department of Veterinary Medicine and Animal Production of the University of Napoli Federico II, Italy (prot. N. 2017/0017676). The trial was carried out in an organic laying hen farm of Avellino (Italy) along 14 weeks, from February to May 2019.

A total of 80 Hy-Line W-36 Single Comb White Leghorn, 18 weeks old hens, weighing an average 1.57 ± 0.09 kg, were homogenously divided into two groups (40 hens per group; 8 replicates of 5 hens each/group). Each group was maintained in a free-range area provided with an indoor box for the night recover of the hens. Each indoor box was 1 × 1 m (l × w), providing an indoor stocking density of 5 hens per square meter, with a deep litter (rice hulls) flooring system. The hens were provided with perches and nest boxes indoors and had access to the external area via pop holes on the side of the shed opposite to the nest. The colony nest used for each indoor shed was 21 cm long and 20 cm wide with one central partition creating 2 compartments of equal size, providing a nest space of 84 cm^2 for each hen. In the free-range area (5 × 5 m), the space available for each hen was around 5 m^2. Each free-range area was enclosed by a 2 m high metal fence protected by a shaded net to deny access to predators. Four "feeding points", containing troughs and nipples for the distribution of fresh water, were organized in each area under a plastic canopy. In the free-range area there were shelters and trees but no grass, so no additional feed was available. The hens were allowed to access to the free-range area at 24 weeks of age, when the target of ~50% of egg production was achieved. After this time, doors of the indoor boxes were manually opened each morning at 08:30 and closed each evening at dusk, once all the hens gained the box area. No further light was supplied in the indoor area. During the experimental period the environmental conditions changed widely both for the temperature (max 15.7 ± 4.3 °C, min 7.0 ± 3.9 °C) and the relative humidity (mean 76.0% ± 13.6). The eggs were collected manually every day. The eggs laid on the floor or on the external area were collected and counted every day.

Table 1
Ingredients and chemical composition of the diets used in the trial.

Ingredients (%)	Ancient Grains diet[a]	Control diet[a]
Maize grain (*Zea mays*)	25.0	59.3
Emmer wheat middling (*Triticum durum dicoccum*)	18.15	–
Common wheat middling (*Triticum aestivum*)	18.15	–
Soybean meal	27.0	30.0
Salt	0.2	0.2
Calcium carbonate	8.0	8.0
Vegetable oil	2.0	1.0
Vitamin-mineral premix	1.0	1.0
Monocalcium Phosphate	0.5	0.5
Chemical characteristics (as feed basis)		
Dry matter[1], %	87.7	88.40
Crude Protein[1], %	17.7	17.2
Ether Extract[1], %	4.20	3.64
Neutral Detergent Fibre[1], %	12.1	9.28
Acid Detergent Fibre[1], %	5.12	4.96
Acid Detergent Lignin[1], %	0.79	0.71
Ca[2], %	3.41	3.42
P[2], %	0.47	0.46
Methionine[2], %	0.60	0.51
Lysine[2], %	1.15	0.96
ME[2], Kcal/kg	2,76	2,73

Vitamin-mineral premix contained the following per kg: retynil acetate 10.000 IU, vit D3 3000IU, vit E 45 mg, Vit B6 4.0 mg, vit b12 0.02 mg, vit k3 3.5 mg, d-pantotenate calcium 13.9 mg, niacin 50 mg, biotina 0.2 mg, ferrous sulphate 122 mg, cupric sulphate 96 mg, Zinc oxide 124 mg, manganese oxide 129 mg, anhydrous calcium iodate 1.5 mg, sodium selenite 0.44 mg.[1] analyzed values; [2]: calculated values.

[a] Celite (20 g/kg) was added to all diets as an indigestible marker in the last 10 days of the trial to each diet at the expense of corn (control diet) and either corn or wheat (ancient grain diets) to measure nutrient digestibility.

2.2. Diets

The two groups were fed once a day two isoprotein and isoenergetic diets, differing in the ingredients as reported in the Table 1. The Control group (C) fed a standard, organic maize-soybean meal diet developed to exceed the hens' requirements indicated in the W-36 commercial layers Management guide (Hi-line, 2016); in the Ancient Grains group (AG), a part of the maize grain (around 57.8%) was replaced by a mix of ancient grains middling, consisting in 50:50 of *Triticum aestivum* var. *spelta* (spelt) and *Triticum durum dicoccum* L. (emmer wheat). In order to avoid possible adverse effects on animal feed choice, maize and wheats middlings were grinded finely to reach the same particle size. An indigestible marker (Celite®, Sigma-Aldrich, St.Louis, Mo) was added at the dosage of 20 g/kg during the last 10 days of the trial to each diet at the expense of corn (control diet) and either corn or wheat (ancient grain diets) to measure nutrient digestibility. The chemical-nutritional characteristics of the diets, cereal sources and ileal content of hens were determined according to the AOAC procedures AOAC, 2005) (ID number: 2001.12, 978.04, 920.39, 978.10 and 930.05 for DM, CP, EE, CF and ash, respectively). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were also determined according to Van Soest et al. (1991). The metabolizable energy of the diets was calculated according to the NRC (1994). The amount of Ca, P, Methionine and Lysine were estimated according to the content of the diet ingredients. The chemical-nutritional characteristics of the grains used in the trial are reported in Table 2.

The amount of acid insoluble ash (AIA) in the diets and in the ileal contents of the hens was measured according to Vogtmann et al.

Table 2
Chemical characteristics of the cereal sources used in the trial (% as feed basis).

Chemical characteristics	Maize grain	Spelt wheat middlings	Emmer wheat middlings
Dry matter[1], %	88.7	87.9	89.2
Ash[1], %	1.3	3.4	2.8
Crude protein[1], %	7.65	13.2	11.8
Ether extract[1], %	3.7	3.5	2.7
Neutral detergent fibre[1], %	8.9	21.7	13.2
Acid detergent fibre[1], %	3.2	5.4	4.3
Acid detergent lignin[1], %	0.9	1.4	1.1
Ca[2], %	0.02	0.04	0.04
P[2], %	0.26	0.47	0.51
ME[2], kcal/kg	3,32	2,87	3,43

1 analyzed values; 2: calculated values.

(1975), using Celite® as an internal marker. Thus, the apparent ileal digestibility coefficients of dry matter, organic matter, crude protein and ether extract of the diets were estimated using the following equation:100–100 × [(% AIA in the diet/% AIA in the ileal content) × (% nutrient in the ileal content/% nutrient in the diet)].

2.3. In vivo evaluations

At 22 weeks of age (when hens were allowed to the external area), the performance monitoring per replicate started. The controls were made along 98 days, from the 13th February to the 11th of May 2019 (14 weeks of laying). The mortality rate was controlled every day and, also, every day the amount of eggs laid in the nests and outside the nests were counted to calculate the laying rate. The feed intake (FI) was recorded every week by weighing the amount of feed administered, residue and scattered by the animals. Every week, 12 eggs per replicate (96 per group) were collected, and stored at 4 °C. For each replicate, the egg mass was calculated as egg weight x egg production percentage and the feed conversion ratio (FCR) was calculated as the grams of daily feed intake divided by the grams of egg weight per day.

2.4. Productive performance

The day after the collection, 6 eggs per replicate (48 eggs per group) were used to measure their physical properties. The eggs length (L), width (W), as well as the eggshell thickness were measured as the average of three equidistant points on the equator of each egg using a micrometer gauge with spherical tips (type 4,139,049, Mitutoyo Inc., Japan). Thereafter, shell, yolk and albumen were separated, weighed and expressed as percentage of the whole egg weight. The egg yolk colour was evaluated visually by using the Roche scale; the pH of the yolk and albumen was measured using a pH-meter (model 720A+ Thermo Fisher Scientific, Rodano, MI, Italy). An electronic breaking strength tester (BMG 1.2mc/D, Messgerätebau Gutsch, Nauendorf) was used to measure eggshell strength.

2.5. Blood analyses

In order to evaluate the effects of the nutritional treatment on animal welfare and health, at 34 weeks of age blood samples were collected from the wing vein of 16 birds per group (2 hens per replicates, a total of 36 blood samples) in plastic tubes with and without sodium heparin as anticoagulant. The whole heparinised blood was used to perform a complete blood cell count (CBC) using an automatic blood analyser (ADVIA 120 Siemens, Munich, Germany). The serum was separated by centrifugation at 1500 $\times g$ for 15 min and stored at −20 °C until the analyses were performed. All biochemical traits of the blood serum — uric acid (UA), creatinine (Crea), total protein (TP), albumin (Alb), globulin (Glob), cholesterol (Cho), triglycerides (Tri), glucose (Glu), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl-transferase (GGT), lactic dehydrogenase (LDH), lactate and creatine kinase (CK) — were determined using commercially available kits from Spinreact (Girona, Spain) by means of enzymatic colorimetric or kinetic methods according to the manufacturer's instructions. In addition, full diagnostics, calibration and running checks were performed by means of specific checks and calibrators (Spintrol H Normal and calibrator, Spintrol B Normal and calibrator) from the same factory. The spectrophotometric measurements were performed using an automatic biochemical analyser, AMS AUTOLAB (Rome, Italy). The globulin concentration was estimated as the difference between total protein and albumin. The albumin to globulin ratio was also calculated.

2.6. Post-mortem evaluations

At the end of the trial (36 weeks of age), 16 hens per group (2 per replicate, 36 hens in total) were slaughtered in a specialized slaughterhouse and the caeca of each hens were tied at both ends, separated by sterile instruments from the rest of the gastrointestinal tract, placed in tightly closed plastic bags and put in pre-warmed thermos. After the sampling, the material was transported as soon as possible (about 1 h) to the laboratory, where two quotes of the caecal content (each about 5 ml) were used for the short chain fatty acids (SCFA) determination. The samples were diluted with oxalic acid (1:1, *v*/v) and SCFA were analyzed by a gas chromatography method (Stanco et al., 2003) (Thermo-Electron mod. 8000top, FUSED SILICA Gaschromatograph, ThermoElectron Corporation, Rodano, Milan, Italy) equipped with an OMEGAWAX 250 fused silica capillary column 30 m × 0.25 mm × 0.25 mm film thickness, flame ionisation detector (185 °C), carrier helium (1.7 ml/min) under isothermal condition (125 °C).

The ileum was dissected from 20 mm after Meckel's diverticulum to 40 mm proximal to the ileocecal junction, in order to avoid the contamination of the other intestinal contents, and the digesta were pooled per replicate (one pool from two hens per replicate; 16 pools per group), immediately frozen and subsequently freeze-dried. The dried ileal digesta was ground enough to pass through a 1-mm sieve and stored at −20 °C until the chemical analysis.

2.7. Statistical analysis

The data were processed using by a one-way ANOVA, using the PROC GLM of SAS (2000) according to the following model:

$$Yij = m + MFi + eij$$

where Y is the single observation, m the general mean, MF the effect of the main factor (i = Control vs. Ancient Grains Diet), e the error. The experimental unit was the replicate. Shapiro-Wilk test was used to check the normal distribution of the data. The comparison between the means was performed using the Tukey's test (SAS, 2000). The results were expressed as mean and the significance level was set at $p \leq 0.05$; P values < 0.10 are considered as a tendency.

3. Results

3.1. In vivo evaluations

Along the trial, nor mortality neither clinical signs of disease were detected for both groups. The laying rate was calculated taking into account also the eggs laid out of the nests. On this regard, the amount of eggs laid out of the nest per day ranged from 14.3 (at the beginning of the trial) to 6.5% at the end of the trial with no differences between the two groups.

Table 3 shows the effect of the dietary treatment on the productive performance of the laying hens. The birds fed the maize based-diet

Table 3
Productive performance of the hens according to dietary treatment.

Productive performance	Ancient grains diet	Control diet	SEM	P value
Initial weight, kg	1.55	1.60	0.007	0.1141
Final weight, kg	1.51	1.53	0.009	0.4916
Laying rate, %	80.16	78.51	1.01	0.7664
Egg weight, g	60.32^B	63.82^A	0.61	< 0.0001
Egg mass, g/hens/day	48.35	50.10	0.33	0.3637
Feed intake, g	120.9^B	134.1^A	1.54	0.0042
FCR, g/g	2.00^b	2.10^a	0.001	0.0428

FCR: feed conversion ratio; SEM: standard error of the mean; a, b: $p < 0.05$; A, B: $p < 0.01$.

showed a higher ($P < 0.01$) egg weight and feed intake than the Ancient Grains group, but the laying rate and the egg mass value were unaffected by the dietary treatment. The feed conversion ratio was more favourable ($P < 0.05$) in the hens fed with the Ancient Grains middlings diet.

3.2. Apparent ileal digestibility

Table 4 reports the apparent ileal digestibility values of dry matter, organic matter, crude protein and ether extract of hens according to the dietary treatments. No differences emerged between groups.

3.3. Productive performance

Table 5 reports the effects of the dietary treatments on the physical characteristics of the eggs produced by the hens. The eggs produced by the hens fed on the Control diet showed higher length and width than the experimental group ($P < 0.01$), while the egg shape was not different between the groups. The shell thickness and strength were higher in the Control group ($P < 0.01$ and $P < 0.05$, respectively). The yolk colour was more red-tending in the Control group ($P < 0.01$).

3.4. Blood analyses

Table 6 shows that there were no significant effects of the dietary treatments on blood cell count of laying hens.

Table 7 shows the effect of the dietary treatments on the biochemical profiles of the laying hens. The total protein, albumin and globulin levels were higher ($P < 0.01$) in the Control group, but the albumin to globulin ratio was higher in the Ancient Grains group. The cholesterol and triglycerides levels were higher ($P < 0.05$) in the hens of the Control group and the same happened for ALT and GGT values ($P < 0.01$).

Table 8 reports the mineral blood profiles of the laying hens according to the dietary treatments. The amount of Ca was higher ($P < 0.01$) in the blood of the hens fed on the Control diet. The levels of P tended ($P = 0.051$) to be higher in the hens from the Control group.

Table 9 reports the effect of the dietary treatments on the SCFA profile of the hen's caecal content. The hens fed on the Ancient Grains diet showed a higher amount of butyrate than the Control group both expressed as absolute ($P < 0.05$) or relative ($P < 0.01$) value.

Table 4
Apparent ileal digestibility of nutrients (%) in hens according to the dietary treatment.

Nutrient digestibility	Ancient grains diet	Control diet	SEM	*P*-value
Dry matter	74.56	75.32	0.31	0.6587
Organic matter	75.12	75.98	0.29	0.7251
Crude Protein	86.23	85.47	0.34	0.6523
Ether extract	92.48	91.87	0.36	0.3954

SEM: standard error of the mean; A, B: $p < 0.01$.

Table 5
Physical characteristics of the eggs according to the dietary treatments.

Egg's characteristics	Ancient grains diet	Control diet	SEM	P value
Egg length, cm	4.59^B	5.10^A	0.015	< 0.0001
Egg width, cm	5.80^B	6.23^A	0.011	< 0.0001
Shape index	1.26	1.22	0.001	0.0869
Shell, %	13.88	14.19	0.24	0.6473
Yolk, %	28.51	28.23	0.31	0.6694
Albumen, %	57.61	57.58	0.75	0.2069
Shell thickness, mm	0.55^B	0.58^A	0.001	0.0166
Shell strength, g/cm^2	4.548^b	5.007^a	8.25	0.0458
Yolk colour	7.96^B	12.23^A	0.19	< 0.0001
Yolk pH	6.53	6.43	0.04	0.4107
Albumen height, cm	1.13	1.14	0.001	0.9162
Albumen pH	8.61	8.69	0.06	0.4448

SEM: standard error of the mean; a, b: $p < 0.05$; A, B: $p < 0.01$.

Table 6
Effect of the dietary treatments on the blood cell count of the laying hens.

Haematological parameters	Ancient Grains diet	Control diet	SEM	P-value
HCT	27.85	30.73	0.27	0.1271
HGB, g/dl	11.93	13.10	0.12	0.1363
RBC, 10^6/mm^6	2.52	2.73	0.01	0.2902
WBC, 10^3/mm^3	30.88	28.67	0.35	0.6191
Heter 10^3/mm^3	6.31	7.26	0.05	0.7847
Lymp, 10^3/mm^3	20.89	19.53	0.28	0.6546
Mono, 10^3/mm^3	2.82	1.77	0.02	0.1136
Eos, 10^3/mm^3	0.48	0.43	0.001	0.6422
Bas, 10^3/mm^3	0.055	0.067	0.001	0.7416
H/L	0.328	0.420	0.001	0.693

HCT: haematocrit; HGB: haemoglobin; RBC: red blood cells; WBC: white blood cells; Heter: heterophils; Lymp: lymphocytes; Mono: monocytes; Eos: eosinophils; Bas: basophils; H/L: heterophils/lymphocytes. SEM: standard error of the mean; a, b: $p < 0.05$.

Table 7
Effect of the dietary treatments on the serum biochemical profile of the laying hens.

Biochemical parameter	Ancient grains diet	Control diet	SEM	P-value
UA, mg/dl	4.99	5.18	0.002	0.8057
Crea, mg/dl	0.45	0.39	0.001	0.2959
TP, g/dl	4.09^B	6.04^A	0.002	< 0.0001
Alb, g/dl	1.36^B	1.82^A	0.001	0.0004
Glob, g/dl	2.72^B	4.22^A	0.001	0.0001
A/G	0.51^a	0.44^b	0.000	0.0324
Chol, mg/dl	107.1^b	126.3^a	1.56	0.0217
Tri, mg/dl	296.8^b	699.3^a	3.39	0.0292
Glu, mg/dl	198.75	202.5	2.05	0.8355
AST, U/L	120.4	145.8	2.56	0.1405
ALT, U/L	133.6^B	317.1^A	1.97	0.0020
GGT, U/L	66.63^B	124.13^A	0.75	0.0028
LDH, U/L	735.25	688.88	4.21	0.6452
CK, U/L	419.13	442.38	3.33	0.7593

UA: uric acid; Crea: creatinine; TP: total proteins; Alb: albumins; Glob: globulins; A/G: albumins/globulins; Chol: cholesterol; Tri: triglycerides; Glu: glucose; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl-transferase; LDH: lactic dehydrogenase; CK: creatine kinase. SEM: standard error of the mean; a, b: $p < 0.05$; A, B: $p < 0.01$.

4. Discussion

The use of ancient wheats in poultry diets as partial substitution of maize reduced the feed intake of the animals withut penalizing the laying rate but decreasing the egg weight. The reduction of the feed intake was probably due to a low palatability of the wheats in

Table 8
Mineral blood profiles of the laying hens.

Mineral blood profile	Ancient grains diet	Control diet	SEM	P-value
Ca, mg/dl	9.83[B]	11.92[A]	0.10	0.0098
P, mg/dl	6.36	7.44	0.05	0.0508
Mg, mg/dl	5.19	5.33	0.03	0.7132
Fe, mcg/dl	205	197.5	1.97	0.7032
Cl, mmol/dl	118.6	110.1	1.25	0.1883

SEM: standard error of the mean; A, B: $p < 0.01$.

Table 9
SCFA profile of the hens caecal content.

SCFA'caecal content	Ancient Grains diet	Control diet	SEM	P value
	Mmol/l			
Acetate	56.32	63.08	0.94	0.2286
Propionate	18.84	20.72	0.25	0.3851
Isobutyrate	1.66	1.91	0.001	0.2778
Butyrate	6.35[a]	5.14[b]	0.01	0.0379
Isovalerate	1.85	1.90	0.002	0.8446
Valerate	11.38	11.89	0.11	0.7315
Total SCFA	96.17	104.6	1.33	0.3481
	% of total SCFA			
Acetate	58.36	60.30	0.87	0.1024
Propionate	19.44	19.64	0.22	0.8433
Isobutyrate	1.71	1.86	0.02	0.4253
Butyrate	6.49[A]	4.92[B]	0.02	< 0.0001
Isovalerate	1.95	1.83	0.01	0.5363
Valerate	12.05	11.44	0.12	0.6226

SCFA: short chain fatty acids; SEM: standard error of the mean; a, b: $p < 0.05$; A, B: $p < 0.01$.

comparison to the maize. Razuki et al. (2018) found a lower feed intake of broilers fed a wheat-based diet compared to a group fed an isoproteic and isoenergetic maize-based diet; however, no data are available in the literature on the palatability of the ancient wheats for poultry. The lower feed intake of the Ancient Grains diet in the hens, not associate with an improved nutrient digestibility as showed by our records, implied a reduction of the digestible protein intake per day: taking into account the protein content of the diets, the average feed intake and the apparent ileal digestibility coefficient of the protein, the hens from AG group consumed 18.45 g of digestible protein by day, while the hens from the Control group 19.70 g. According to our results, other authors (Saikhlai et al., 2019) found similar dry matter and organic matter digestibility by feeding laying hens with wheat (in ratio of 5, 10, 15, 20, or 25% in replacement to maize). By contrast, Choct et al. (1996) reported that the ileal digestibility of starch, protein, and lipids was significantly depressed in broilers when wheat by-products were included in the diet, probably due to the high levels of non-starch polysaccharide as structural carbohydrate in the aleurone and endosperm walls. Also, lipids must be emulsified before digestion and absorption, and such process is hindered by viscous digesta (Mirzaie et al., 2012). However, in our trial no differences between the treatments were observed both for transit time and moisture of faeces.

It is well known that the egg size usually increases according to the increase of the protein intake of hens (Gunawardana et al., 2008; Shim et al., 2013). Despite that, since the laying rate was slightly higher (even if not significantly different) than the Control group, the egg mass was not different between the two dietary treatment and the feed conversion ratio resulted more favourable in the hens fed on the Ancient Grains.

According to Ketta and Tumova (2018), the higher egg weight detected in the Control group was accompanied by a higher eggshell thickness and strength. Studying the Pearson's coefficients between eggshell parameters in hens farmed on litter, such authors showed a positive correlation between egg weight and eggshell strength ($P < 0.01$) as well as between egg weight and eggshell thickness ($P < 0.001$).

A role on the differences of eggshell strength and thickness values between the two groups could also be played by the different levels of Ca and P in hen's blood. The nutritional role of calcium is closely linked to that of phosphorus and to vitamin D effect (Neijat et al., 2011). The metabolic and structural function of both these minerals is essential for laying hen's production because they are responsible of the eggshell formation (Graveland, 1996). According to Berne and Levy (1998) Ca is actively absorbed in all intestinal segments (in particular, in the duodenum and the jejunum), but Ca absorption depends on animal nutritional status. Indeed, in birds fed low calcium diets its absorption increases, whereas high dietary levels of this mineral reduce its absorption. At least two hypotheses can be considered to explain the differences between groups on the eggshell physical properties. First, we must consider the lower feed intake of the hens fed ancient wheats diet. Considering the amount of Ca and P in the diets and the average daily feed intake, the hens from the Control group ingested around 4.59 g of Ca and 0.62 g of P by day, while the hens from the Ancient Grains group ingested 4.12 g of Ca and 0.57 g of P by day. Another consideration can be made on the P availability of the diets. Considering that the supplementation of Ca and P in the diets was the same, we should analyse the raw materials and the cereals used in this trial. In fact, the availability of P for poultry in the maize is an average 21%, while in wheats it is 41%, according to Huang and Allee (1981) and Coffey et al. (1994). Therefore, the available P ingested by day from cereal sources was 0.05 g for the Control group and 0.11 g for the Ancient Grains group. It is therefore possible that the higher P available in the Ancient Grains group could form an insoluble calcium phosphate complex in the intestine, which made the Ca unavailable for digestion (de Vries et al., 2010).

The results on serum cholesterol levels revealed that there was a high reduction of the cholesterol in the Ancient Grains group as compared to the Control. Some authors reported a positive correlation between calcium and cholesterol in serum (Sloan et al., 1994). In most animals, cholesterol is eliminated by catabolism and excretion in the faeces as bile acids, but the hens eliminate considerable amounts of cholesterol in the eggs. For this reason, the study of diets that are able to reduce the level of cholesterol, both in blood and in eggs, seems very important in this species (Kurtoglu et al., 2004). The diets tested in our trial are different in terms of fiber constituents, but these differences must be well analyzed. The Ancient Grains diet contained +30.4% of neutral detergent fiber (NDF) than the Control and only +3.2% of acid detergent fiber (ADF). As the hemicellulose content can be calculated as NDF – ADF (Rinne et al., 1997), it results that AG diet contains +61.6% of hemicellulose than the Control diet. In broilers, a negative correlation has been demonstrated between dietary fiber content and cholesterol in serum (Sarikhan et al., 2009). Researches in pigs (Kreuzer et al., 2002) showed that hemicelluloses have a more favourable effect of decreasing blood cholesterol than hardly digestible fiber, fermentable cellulose or pectin. Varastegani and Dahlan (2014) showed that the increase of the amount of dietary fiber in the diet leads to significant impact on biochemical parameters, including total cholesterol and triglycerides. Triglycerides present in serum are of exogenous and endogenous origin. The available literature provides few data on the content of triglycerides in the serum of poultry. In mammals, bile lipids are mainly composed by phospholipids and cholesterol whereas in poultry they are constituted by cholesterol esters and triglycerides. Leeson et al. (1997) hypothesized that a regulatory effect due to fiber content should exist for these lipids, thus, the reduction of triglyceride levels in the Ancient Grains group may be due to the ability of fiber to bind with the lipid structure.

Serum enzymes can provide information of the degree of organs or tissue damage. The concentration in serum of liver enzymes, such as AST, ALT and GGT, can be used to evaluate avian hepatic function because their synthesis occurs in the liver, but it is assumed that high activity of these enzymes can indicate organ damage (González and

Silva, 2006). In addition, the liver contributes to the synthesis of proteins, enzymes and hormones, some of them playing a key role in bone formation and Ca metabolism (Jiang et al., 2013) with consequent effects on eggs. In this trial, we observed significantly higher hepatic enzymes in the hens fed on the Control diet compared to the Ancient Grains diet. As also reported by other authors, laying hens fed diets containing wheat instead of maize showed a better hepatic function (Jensen et al., 1974; Traineau et al., 2013). Therefore, we can assume that not only the energy content, but also some dietary factors, can be involved in ensuring an optimal liver function. This suggests that maize might either contain or lack factors which affect liver physiology in laying hens, but such hypothesis needs further specific studies to be confirmed.

Differences in serum protein are not always easy to explain. The Control group showed higher levels of TP, albumin and globulin, but such differences resulted in a lower A/G ratio respect to the Ancient Grains fed group. Griminger and Scanes (1986) showed that high globulin levels and low albumin/globulin ratios indicate an improvement of immune response and, therefore, a better disease resistance in birds. Since blood proteins in birds are important indicators for the evaluation of health status and the most important increase in this trial involved globulins, deeper studies performing a protein profile including the concentrations of serum protein fractions are needed to clarify the obtained results.

The more intensive red colour of yolk in the eggs from the Control group, is related to the higher level of maize inclusion in the diet. According to the USDA National Nutrient Database (2020) soft and hard white wheats as well as red wheats, contain 2.2 μg/g DM of lutein and zeaxanthin, while maize, which is the richest source of lutein among the cereal grains, contains around 13.6 μg/g DM of lutein and zeaxanthin. Çiftci et al. (2003) found lower egg yolk colour (6.30 Roche colour scale) in wheat-based diets compared to maize diet (8.70 Roche colour scale) in Hisex Brown laying hens (16 to 27-weeks-old). Similar results were found by Abudabos (2011), who observed a higher redness of yolk in Hy-Line hens (36 to 51-weeks-old) fed a maize-soy diet compared to wheat middlings-containing diets.

In the literature, few data are available on the effect of ancient grains on short fatty acid volatile production. The higher amount of butyric acid in the caecal content of hens fed the Ancient Grains detected in our trial is in line with the findings of other authors comparing the differences between corn and common wheats.

Masey-O' Neill et al. (2014) observed contrasting cereal grain effects at 49 d of age with broilers fed wheat-based diets exhibiting higher acetic, butyric, caproic, and total SCFA, but lower propionic concentrations when compared with those fed the maize-based diets. Kiarie et al. (2014) found higher concentrations of acetic and butyric acids in male broiler chicks of 21 weeks of age fed wheat compared to maize diets. Also, McCafferty et al. (2019) found a higher ($P < 0.01$) production of butyric acid in the caecal content of broiler fed wheat at 40 days of age in comparison to the maize diet. The butyric acid is considered the main enterocytes energy source (Bovera et al., 2010) and is also necessary for a proper development of the Gut-Associated Lymphoid Tissue (Mroz, 2005). This is important for maintaining the function of the entire gastro-intestinal tract, not just of the caecocolon (Montagne et al., 2003). When a higher amount of butyric acid is available, the increase of the nutrients for enterocytes enhances the flow of blood through the intestine and then the tissue oxygenation as well as nutrient transport and absorption (Mahdavi and Torki, 2009). In humans, Saemann et al. (2000) demonstrated an anti-inflammatory property of the butyrate, which may have great implications for the regulation of the immune response, while the association of the butyrate with the immune and anti-inflammatory response in poultry is limited (Bedford and Gong, 2018). van Der Wielen et al. (2000) reported that the volatile fatty acids exerted a bacteriostatic effect against some enteric bacteria, including *Salmonella typhimurium*, without inhibiting the beneficial bacteria of the gastrointestinal tract, such as *Lactobacillus*, in chickens. In addition, the same authors found a negative correlation between the acetate levels and CFU of Enterobacteriaceae (including Salmonella spp.) in broilers.

5. Conclusions

The Ancient grain diet showed a more favourable feed conversion ratio. Importantly, the results on blood parameters suggest that such diet not only had no negative effects on hens health but it may have beneficial effects showing lower values of liver specific enzymes (i.e. ALT and GGT) as well as of cholesterol and triglycerides. The higher amount of butyric acid in the caecal content of hens fed the Ancient Grains diet suggests an improvement of the nutrient transport and absorption, maintaining the function of the entire gastro-intestinal tract, not just of the caeco-colon. The Ancient Grains diet only affected some eggs characteristics (thickness, length, and weight) with no negative effects in commercial terms. Thus, the Ancient Grains diet may guarantee production performances with positive effects on animal health and on the environmental sustainability, because of its ability to growth in the marginal areas. Future studies should address the possible effects of the Ancient Grains diet of eggs quality in terms of nutritional value.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

Abudabos, A.M., 2011. Effect of enzyme supplementation and wheat middlings as an alternative to corn on laying hens performance. It. J. Anim. Sci. 10 (e57), 254–259. https://doi.org/10.4081/ijas.2011.e57.

AOAC, 2005. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Arzani, A., 2011. Emmer (Triticum turgidum ssp. dicoccum) flour and bread. In: Preedy, V.R., Watson, R.R., Patel, V.B. (Eds.), Flour and Fortification in Health and Disease Prevention. Elsevier, Academic Press Imprint, Amsterdam, pp. 69–78.

Bedford, A., Gong, J., 2018. Implications of butyrate and its derivatives for gut health and animal production. Animal nutrition (Zhongguo xu mu shou yi xue hui) 4 (2), 151–159. https://doi.org/10.1016/j.aninu.2017.08.010.

Benincasa, P., Galieni, A., Manetta, A.C., Pace, R., Guiducci, M., Pisante, M., Stagnari, F., 2015. Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species. J. Sci. Food Agric. 95, 1795–1803. https://doi.org/10.1002/jsfa.6877.

Berne, M.R., Levy, M.N., 1998. Fisiologia, 4th ed. Guanabara Koogan, Rio de Janeiro.

Boukid, F., Folloni, S., Sforza, S., Vittadini, E., Prandi, B., 2018. Current trends in ancient grains-based foodstuffs: insights into nutritional aspects and technological applications. Compr. Rev. Food Sci. Food Saf. 17, 123–136. https://doi.org/10.1111/1541-4337.12315.

Bovera, F., Marono, S., Di Meo, C., Piccolo, G., Iannaccone, F., Nizza, A., 2010. Effect of mannanoligosaccharides supplementation on caecal microbial activity of rabbits. Animal 4, 1522–1527. https://doi.org/10.1017/S1751731110000558.

Ceccarelli, S., Grando, S., 1996. Drought as a challenge for the plant breeder. Plant Growth Regul. 20, 149–155. https://doi.org/10.1007/BF00024011.

Chandi, G.K., Lok, C.W., Jie, N.Y., Seetharaman, K., 2015. Functionality of Kamut and millet flours in macro-wire-cut cookie systems. J. Food Sci. Technol. 52, 556–561. https://doi.org/10.1007/s13197-013-0969-z.

Choct, M., Hughes, R., Wang, J., Bedford, M., Morgan, A., Annison, G., 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non starch polysaccharides in chickens. Br. Poult. Sci. 37, 609–621. https://doi.org/10.1080/00071669608417891.

Çiftci, I.E., Yenice, A., GökÇeyrek, D., Öztürk, E., 2003. Effects of energy level and

enzyme supplementation in wheat-based layer diets on hen performance and egg quality. Acta Agric. Scand. Sec. A 53, 113–119. https://doi.org/10.1080/09064700310017967.
Coffey, R.D., Mooney, K.W., Cromwell, G.L., Aaron, D.K., 1994. Biological availability of phosphorous in defluorinated phosphates with different phosphorous solubilities in neutral ammonium citrate for chicks and pigs. J. Anim. Sci. 72, 2653–2660. https://doi.org/10.2527/1994.72102653x.
de Vries, S., Kwakkel, R.P., Dijkstra, J., 2010. Dynamics of calcium and phosphorous metabolism in laying hens. In: Vitti, D.M.S.S., Kebreab, E. (Eds.), Phosphorous and Calcium Utilization and Requirements in Farm Animals. Pub: CAB International, pp. 133–150.
Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., Sofi, F., 2018. Ancient wheat species and human health: biochemical and clinical implications. J. Nutr. Biochem. 52, 1–9. https://doi.org/10.1016/j.jnutbio.2017.09.001.
Fardet, A., 2014. How can both the health potential and sustainability of cereal products be improved? A French perspective. J. Cereal Sci. 60, 540–548. https://doi.org/10.1016/j.jcs.2014.07.013.
Giambanelli, E., Ferioli, F., Kocaoglu, B., Jorjadze, M., Alexieva, I., Darbinyan, N., Antuono, F., 2013. A comparative study of bioactive compounds in primitive wheat populations from Italy, Turkey, Georgia, Bulgaria and Armenia. J. Sci. Food Agric. 93, 3490–3501. https://doi.org/10.1002/jsfa.6326.
González, F.H.D., Silva, S.C., 2006. Perfil bioquímico sanguine. In: González FHD and S.C. Silva Introdução à Bioquímica Clínica Veterinária. Brazil: UFRGS 313–358.
Graveland, J., 1996. Avian eggshell formation in calcium-rich and calcium-poor habitats: importance of snail shells and anthropogenic calcium sources. Can. J. Zool. 74, 1035–1044. https://doi.org/10.1139/z96-115.
Griminger, P., Scanes, C.G., 1986. Protein metabolism. In: Avian Physiology, 4th Edn. P. D. Sturkie. Ed. Springer Verlag, New York, pp. 326–345.
Gunawardana, P., Roland, D.A., Bryant, M.M., 2008. Effect of energy and protein on performance, egg components, egg solids, egg quality, and profits in molted Hy-line W-36 hens. J. Appl. Poult. Res. 17, 432–439. https://doi.org/10.3382/japr.2007-00085.
Huang, K.C., Allee, G.L., 1981. Bioavailability of phosphorous in selected feedstuffs for young chicks and pigs. J. Anim. Sci. 53, 248–252.
Hy-Line, 2016. W-36 Commercial Layers Management guide. available at. https://www.hyline.com/UserDocs/Pages/36_COM_ENG.pdf.
Jensen, L.S., Falenand, L., Chang, C.H., 1974. Effect of distillers dried grains with solubles on reproduction and liver fat accumulation in laying hens. Poult. Sci. 53, 586–592. https://doi.org/10.3382/ps.0530586.
Jiang, S., Cheng, H.W., Cui, L.Y., Zhou, Z.L., Hou, J.F., 2013. Changes of blood parameters associated with bone remodelling following experimentally induced fatty liver disorder in laying hens. Poult. Sci. 92, 1443–1453. https://doi.org/10.3382/ps.2012-02800.
Ketta, M., Tumova, E., 2018. Relationship between eggshell thickness and other eggshell measurements in eggs from litter and cages. It. J. Anim. Sci. 17, 34–239. https://doi.org/10.1080/1828051X.2017.1344935.
Kiarie, E., Romero, L.F., Ravindran, V., 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler. Poult. Sci. 93, 1186–1196. https://doi.org/10.3382/ps.2013-03715.
Kreuzer, M., Hanneken, H., Wittmann, M., Gerdemann, M.M., Machmuller, A., 2002. Effects of different fibre sources and fat addition on cholesterol and cholesterol-related lipids in blood serum, bile and body tissues of growing pigs. J. Anim. Physiol. Anim. Nutr. 86, 57–73. https://doi.org/10.1046/j.1439-0396.2002.00358.x.
Kurtoglu, F., Kurtoglu, V., Nizamlioglu, M., 2004. Egg and serum cholesterol concentrations and zootechnical performances of layer hens fed with various levels of niacin. Revue Méd. Vét. 155, 393–400.
Leeson, S., Zubair, A.K., Squires, E.J., Forsberg, C., 1997. Influence of dietary levels of fat, fiber and copper sulfate and fat rancidity on cecal activity in the growing Turkey. Poult. Sci. 76, 59–66. https://doi.org/10.1093/ps/76.1.59.
Mahdavi, R., Torki, M., 2009. Study on usage period of dietary protected butyric acid on performance, carcass characteristics, serum metabolite levels and humoral immune response of broiler chickens. J. Anim. Vet. Adv. 8, 1702–1709.
Masey-O' Neill, H.V., Singh, M., Cowieson, A.J., 2014. Effects of exogenous xylanase on performance, nutrient digestibility, volatile fatty acid production and digestive tract thermal profiles of broilers fed on wheat-or maize-based diet. Br. Poult. Sci. 55, 351–359. https://doi.org/10.1080/00071668.2014.898836.
McCafferty, K.W., Bedford, M.R., Kerr, B.J., Dozier, W.A., 2019. Effects of cereal grain source and supplemental xylanase concentrations on broiler growth performance and cecal volatile fatty acid concentrations from 1 to 40 d of age. Poult. Sci. 98, 2866–2879. https://doi.org/10.3382/ps/pez032.
Migliorini, P., Spagnolo, S., Torri, L., Arnoulet, M., Lazzerini, G., Ceccarelli, S., 2016. Agronomic and quality characteristics of old, modern and mixture wheat varieties and landraces for organic bread chain in diverse environments of northern Italy. Eur. J. Agron. 79, 131–141. https://doi.org/10.1016/j.eja.2016.05.011.
Mirzaie, S., Zaghari, M., Aminzadeh, S., Shivazad, M., Mateos, G.G., 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. Poult. Sci. 91, 413–425. https://doi.org/10.3382/ps.2011-01686.
Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108, 95–117. https://doi.org/10.1016/S0377-8401(03)00163-9.
Mroz, Z., 2005. Organic acids as potential alternatives to antibiotic growth promoters for pigs. In: Ball, R.O., Zijlstra, R.T. (Eds.), Advance in Pork Production. University of Alberta Department of Agricultural, vol. 16. Food and Nutritional Science, Edmonton, AB, Canada, pp. 169–182.
Neijat, M., House, J.D., Guenter, W., Kebreab, E., 2011. Calcium and phosphorus dynamics in commercial laying hens housed in conventional or enriched cage systems. Poult. Sci. 90, 2383–2396. https://doi.org/10.3382/ps.2011-01401.
NRC, 1994. Nutrient Requirements of Poultry, 9 rev. ed. Natl. Acad. Press.
Pettersson, I.C., Weeks, C.A., Wilson, L.R.M., Nicol, C.J., 2016. Consumer perceptions of free-range laying hen welfare. Br. Food J. 118, 1999–2013. https://doi.org/10.1108/BFJ-02-2016-0065.
Randall, P., Johnson, Q., Verster, A., 2012. Fortification of wheat flour and maize meal with different iron compounds: results of a series of baking trials. Food Nutr. Bull. 33, S344–S359. https://doi.org/10.1177/15648265120334s311.
Razuki, W.M., Abed, M.K., Al-Naif, H.H.N., 2018. Effects of self-selection diets differing in cereal source and protein level on broiler performance. Int. J. Poult. Sci. 17, 479–485. https://doi.org/10.1038/s41430-017-0044-3.
Rinne, M., Huhtanen, P., Jaakkola, S., 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67, 19–35. https://doi.org/10.1016/S0377-8401(96)01142-X.
Saemann, M.D., Bohmig, G.A., Osterreicher, C.H., Burtscher, H., Parolini, O., Diakos, C., Stock, J., Hor, W.H., Zlabinger, J.G., 2000. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. FASEB J. 14, 2380–2382. https://doi.org/10.1096/fj.00-0359fje.
Saikhlai, K., Poeikhampha, T., Bunchasak, C., Krutthai, N., Chomtee, B., Rakangthong, C., 2019. Effect of whole wheat levels in diet on production performance, egg quality and Nutrient digestibility of laying hens. Indian J Anim Res. 53 (11), 1480–1484. https://doi.org/10.18805/ijar.B-803.
Sarikhan, M., Shahriyar, H.A., Nazer_Adl, K., Gholizadeh, B., Beheshti, B., 2009. Effects of insoluble fiber on serum biochemical characteristics in broiler. Int. J. Agric. Biol. 11 (7), 73–76.
SAS. Statistical Analyses System, 2000. SAS/STAT Software, Version 9. SAS Institute Inc., Cary, NC, USA.
Shewry, P.R., 2018. Do ancient types of wheat have health benefits compared with modern bread wheat? J. Cereal Sci. 79, 469–476. https://doi.org/10.1016/j.jcs.2017.11.010.
Shim, M.Y., Song, E., Billard, L., Aggrey, S.E., Pesti, G.M., Sodsee, P., 2013. Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poult. Sci. 92, 2687–2696. https://doi.org/10.3382/ps.2012-02569.
Sloan, D.R., Harms, R.H., Russell, G.B., Smith, W.G., 1994. The relationship of egg cholesterol to serum cholesterol, serum calcium, feed consumption, and dietary cholecalciferol. Poult. Sci. 7, 472–475. https://doi.org/10.3382/ps.0730472.
Stanco, G., Di Meo, C., Piccolo, G., Nizza, A., 2003. Effect of storage duration on frozen inoculum to be used for the in vitro gas production technique in rabbit. It. J. Anim. Sci. 2, 265–270. https://doi.org/10.4081/ijas.2003.265.
Traineau, M., Bouvarel, I., Mulsant, C., Roffidal, L., Launay, C., Lescoat, P., 2013. Effects on performance of ground wheat with or without insoluble fiber or whole wheat in sequential feeding for laying hens. Poult. Sci. 92, 2475–2486. https://doi.org/10.3382/ps.2012-02871.
U.S. U.S. Department of Agriculture (USDA), 2020. National Nutrient Database. available at:. https://fdc.nal.usda.gov/download-datasets.html.
van Der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A., van Knapen, F., 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl. Environ. Microbiol. 66, 2536–2540. https://doi.org/10.1128/aem.66.6.2536-2540.2000.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
Varastegani, A., Dahlan, I., 2014. Influence of dietary Fiber levels on feed utilization and growth performance in poultry. J. Anim. Sci. Adv. 4, 422–429.
Vogtmann, H., Frirter, P., Prabuck, A.L., 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets Br. Poult. Sci. 16, 531–534.
Whittaker, A., Dinu, M., Cesari, F., Gori, A.M., Fiorello, C., Becatti, M., Casini, A., Marcucci, R., Benedettelli, S., Sofi, F., 2017. A khorasan wheat-based replacement diet improves the risk profile of patients with type 2 diabetes mellitus (T2DM): a randomize crossover trial. Eur. J. Nutr. 56, 1191–1200. https://doi.org/10.1007/s00394-016-1168-2.