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The Metabolizable Energy Evaluation of Rapeseed-Containing Diets in Broiler Chickens Using the Regression Method

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ABSTRACT:
The present research aims to determine the chemical composition, apparent metabolizable energy (AME), Nitrogen-corrected apparent metabolizable energy (AMEn), true metabolizable energy (TME), and nitrogen-corrected true metabolizable energy (TMEn) in rapeseed specimens, and to predict their energy content using regression prediction equations. 30 rapeseed specimens were provided from different animal and poultry feed factories across Iran. The different energy content of each rapeseed specimen was measured in 4 replicates using the force-feeding method. The results of the laboratory analysis of the specimens were as follows: average raw dry matter (89.91%), crude protein (9.25%), crude fat (3.18%), crude fiber (3.83%), ash (1.35%), starch (65.23%), and glucose (9.69%). The results of field experiments were as follows: average raw energy (4365 kcal/kg), AME (3274), AMEn (3209), TME (3863), and TMEn (3771). Using the obtained data, regression equations were fitted to predict AME, AMEn, TME, and TMEn based on chemical compositions. The results showed that rapeseed specimens from different origins were different in raw energy, crude protein, crude fiber, and ash, but no difference was observed between them in crude fat and TMEn. The GPR model showed a favorable performance based on the evaluation criteria of R^2=0.91 and RMSE= 33.67 kcal/kg). The GPR model may improve the ability and capacity to accurately predict the energy of diets to achieve optimal performance in poultry nutrition.

Keywords: Metabolizable energy, Rapeseed, Model prediction, Regression equation

INTRODUCTION:

Nowadays, the increased animal production, especially in developing countries, requires research to find new high-quality food resources. Various feed resources in these areas have not been used optimally, properly, and completely due to insufficient information on their compounds (25). The high cost of feeding, which accounts for 60-70% of the current cost of production makes it necessary to break down feeds to meet the needs of livestock and poultry without additional feed consumption. It is not possible and cost-effective to determine the chemical composition of the feeds used for livestock and poultry breeding units due to the lack of access to dedicated laboratories. So, as a solution to this problem, it is helpful to develop chemical composition tables for the feeds used in the country or different regions with similar weather and climatic conditions and soil characteristics (2, 3, 4). To reach cost-effective production, it is necessary to provide feedstuffs, especially low-cost sources of protein and energy according to the needs of poultry. In this regard, the provision of protein sources faces more restrictions and is usually more expensive than energy sources (16). Rapeseed as a new protein source less expensive than fish and soybean meals has been considered in poultry diets. Moreover, In recent years, rapeseed meal varieties with low erucic acid and glucosinolates have received considerable attention (32, 35).

Today, inadequate feed resources (energy and protein) are considered the most important factor limiting livestock and poultry production in many countries, including Iran. However, nutritional disorders and the imbalance between nutrients in the feed consumed by livestock also play a crucial role in this area. In addition to inadequate resources, the high level of one or more elements in the feed resources of a region may play a role in keeping the production low, however, there is no information on how and the extent to which this issue influences (6). Therefore, examining feedstuffs used in animal nutrition, as a basic area, will play a key role in the development of livestock production and the optimal use of potential resources and animal products (1). Numerous rapeseed hybrids are available due to various environmental factors such as climate change and growing season length, making each grain contain different nutritional values depending on environmental conditions, soil fertility, specific hybrids, etc. The accurate formulation of low-cost diets requires knowing the exact nutritional value of feed. Nutritional assessment is mainly based on the energy and protein (amino acids) nutritional status assessment. These are considered costly nutrients and form small portions of the diet. In most regions of the United States, poultry feed is the lowest energy source,

mainly due to its abundance, low cost, and high digestibility. The change in material composition caused by genetic, agricultural, and management factors has become a concern of nutritionists in the poultry industry. Nutritionists usually deal with the amounts of chemical compositions of feedstuffs in the formulation of low-cost diets considering the time and cost required for analyzing amino acids and important nutrients (13). Today, several high-yielding rapeseed cultivars with a wide range of traits and seed chemical compositions have been developed through genetic advancements. In North America and Western Asia, rapeseeds are commonly used as the main energy source for poultry, accounting for 40-60% of their diet. One of the important measures for the quality of a feedstuff is the nitrogen-corrected true metabolizable energy (TMEn). Rapeseeds have a larger size and a greater specific weight than other cereals, such as barley, soybean, and sorghum despite being similar to them in many other physical and chemical properties. Lack of moisture during growth may result in smaller seeds. The cold can produce seeds with low density. Different soil nitrogen levels or genetic differences can make the endosperm hard or soft and different in density. Therefore, it contains the complete embryo and the structural, nutritional, and enzyme systems needed to start the growth and development of the embryo. Rapeseeds take the top rank among cereals in terms of providing energy, and have a low level of indigestible shell fiber (2%) compared to other cereals, leading to their enhanced significance for feeding livestock and poultry (5). Energy is an important part of poultry feed and is mainly obtained from cereals. Cereals are the main energy source in the poultry diet and account for the largest share of the poultry diet. Among cereals, rapeseed is the main foodstuff used in poultry diets mainly for its high energy content and lack of anti-nutritional substances (13, 41).

In nutritional status assessment, prediction equations can be a useful and desirable tool for estimating digestible energy (DE) and metabolizable energy (ME) concentrations in feed ingredients based on chemical composition (15). Unlike crude protein, crude fat, crude fiber, calcium, and phosphorus, the metabolizable energy analysis of feedstuffs is costly and time-consuming and requires biological experiments. Therefore, it is applicable and of great importance to predict the metabolizable energy of produced feeds based on their chemical compounds (11). The energy content of cereals can be expressed in the form of apparent metabolizable energy (AME), metabolizable energy (ME), and true metabolizable energy (TME). AME is the difference between the animal's energy expenditure and energy loss. TME ……., so its value is always higher than the value of AME. AME and TME can also be corrected to zero nitrogen retention, denoted as AMEn and TMEn. ……. Nitrogen is excreted in the form of uric acid, which contains energy. The amount of uric acid increases as the amount of nitrogen excreted increases. ….. (17). Prediction models are accepted as an information tool to support rapid and cost-effective feedstuff evaluation. The present study aimed to evaluate prediction models in the prediction of metabolizable energy of rapeseed-containing diets in broiler chickens, as well as to investigate the GPR model's ability to predict the TMEn content of rapeseed specimens (as the output of the model) according to their chemical composition (as the input of the model).

MATERIALS AND METHODS:

Experiment time and location:

Rapeseed and excreta specimens were approximately decomposed to determine their chemical composition and raw energy. The experiments were conducted in the quality control laboratory of the Faculty of Agriculture, Urmia University in November 2022. Field experiments and energy measurements were performed with the force-feeding method at the Poultry Research Center of the Faculty of Agriculture. In these experiments, 30 rapeseed specimens were collected from different animal and poultry feed factories across Iran (Tehran, Qom, Karaj, Isfahan, West Azarbaijan, Khuzestan, Kermanshah, and Razavi Khorasan).

Then, about 20 gr of each specimen was separated to measure dry matter, fiber, crude fat, crude protein, crude energy, ash, starch, and glucose. In this experiment, nutrient analysis was performed according to the proposed standard methods, and the amount of nutrients was determined and standardized in the percentage of dry matter.

Table 1- The basic diet before the TMEn test in the 3-week adaptation phase for the tested roosters

Compound	***%***
Rapeseed	*60.9*
Soybean oil	*1.63*
Soybean meal	*34.02*
Dicalcium phosphate	*2.18*
Limestone	*0.8*
Sodium chloride	*0.39*
Vitamins and minerals	*0.31*
Lysine hydrochloride	*0.08*
Methionine	*0.07*
Computational analysis	
AME kcal/kg	*2930*
Crude protein	*19.19*
Calcium	*0.87*
Phosphorus	*0.44*
Lysine	*1.12*

Each kilogram diet included: 0.3 mg cobalt, 5 mg copper, 25mg iron, 1 mg iodine, 125 mg Manganese, 60 mg zinc, 648 mg choline chloride, 3.11 mg trans-retinol, 60 μg Cholecalciferol, 60 mg alpha DL-tocopheryl acetate, 4 mg Menadione, 3 mg thiamine, 12 mg riboflavin, 36 mg niacin, 12.79 mg Calcium

pantothenate, 10 mg pyridoxine, 0.019 mg Cyanocobalamin, 5.11 mg folic acid, 0.21 mg Biotin, 100 mg Antioxidant, 0.5 mg Molybdenum, and 200 μg Selenium.

Measurement of chemical composition:

Dry matter: 2 gr of the specimens were weighed and poured into 5 × 10 cm aluminum containers. The containers were placed in an oven at 80 °C for 24 hours. Next, they were weighed again and the amount of dry matter was calculated using the following equation.

Ash: the specimens were first burned by flame and then, placed in an electric furnace at 600 °C for 6 hours. Next, they were weighed and the amount of ash and organic matter was calculated.

Crude fat: The amount of crude fat in food was measured through the Soxhlet procedure using Tecator Soxtec System HT 1043 extraction unit and diethyl ether solvent. For this purpose, 2 gr of the specimen was weighed with a digital scale with an accuracy of 0.001 ± 0 gr, wrapped in filter paper, placed in the extraction thimble, and transferred into the extraction unit. Two-thirds of the unit's flasks were filled with diethyl ether and the specimens were immersed in ether for 1 h. Next, the specimens were removed from the ether and distilled for 15 minutes. Then, they were placed in an oven for 24 hours. The increase in the flask weight was due to the deposition of fat inside it, which is divided by the specimen weight to calculate the fat content in percent.

Crude protein: The Kjeldahl method was used to measure crude protein. For this purpose, 0.3 gr of the specimen was weighed with a digital scale with an accuracy of 0.001 ± 0 gr and poured into special tubes. Then, 10 cc of concentrated sulfuric acid and a catalyst tablet (4*So2K* ،*Se*) were added. Next, it was placed in the Kjeldahl apparatus at 420 °C for 3 hours. After the digestion process, the specimen was cooled and 20 cc of distilled water was added to it. Next, the nitrogen content (in %) of the specimen was determined using an Automatic Kjeldahl Analyser 1030. The nitrogen content in food is converted to crude protein by a factor of 6.23.

Raw fiber: A Fiber System 110 was used to measure raw fiber. In this method, 2 gr of the specimen was weighed with a digital scale with an accuracy of 0.001 ± 0 grams and poured into crucibles. The crucibles were placed on the abovementioned apparatus, 150 cc of 0.7% sulfuric acid was added to each specimen, and the specimens were boiled for 30 minutes. Then, the specimens were filtered and washed with boiling water. Next, 150 cc of 1.3% caustic soda (sodium hydroxide) was added and the specimens were boiled for 30 minutes. Next, after draining the caustic soda and washing the specimens with warm water, they were removed from the apparatus and cooled in a desiccator. Next, they were burned in an oven at 450-500 °C. The difference between the weight of the specimen before and after burning shows the amount of raw fiber in the specimen. The percentage of raw fibers is determined by dividing its weight by the specimen weight.

Raw energy: The PARR 1261 bomb calorimeter apparatus was used to determine the raw energy content. 1 gr of the specimen was weighed with a digital scale with an accuracy of 0.001 ± 0 gr, made into a pellet using a pelletizing device, and placed in a special steel container. Then, 10 cm of firing wire (chromium-nickel alloy) was cut and connected to the electrodes in such a way that the wire was in contact with the specimen. The specimen was placed inside the bomb calorimeter and 30 atmospheres of oxygen were injected into the bomb. Then, the bomb was placed in the jacket, the electrodes were connected to it, and the jacket was switched on. After the burning process, the bomb was removed and opened to measure the amount of wire used. After washing the contents of the bomb with distilled water, they were poured into an Erlenmeyer flask, and by adding 1 cc of methyl orange to the Erlenmeyer flask, its contents were titrated with 0.071% normal sodium carbonate. Each centimeter of alloy wire produces 23 kilocalories of energy after getting burned.

Glucose extraction: approximately 0.2 gr of the well-ground specimen was weighed with a digital scale with an accuracy of 0.001±0 gr, poured into a 50 ml centrifuge tube, and two drops of 80% ethanol were added. They were mixed well, and then, 5 ml of distilled water was added and the solution was shaken completely. Then, 25 ml of 80% ethanol was added to it. It was shaken vigorously, left for 5 minutes, and centrifuged at 3000 rpm to extract the alcoholic extract. This process was followed by adding 30 ml of 80% ethanol to the remaining specimen and the obtained solution was centrifuged again. The two extracts obtained were combined. The alcohol in the mixture of the two extracts interferes with the reaction between sugar and anthrone, which must be evaporated by a hot water bath at 90 °C. The remaining solution was diluted with water to produce a concentration of 100 μg of sugar per liter of the extract. After this process, the solution was ready to be decomposed with anthrone reagent.

Starch extraction: The remaining specimen from the sugar extraction stage was mixed with 5 ml of distilled water in a 50 ml centrifuge tube. While shaking, 6.5 ml of 52% perchloric acid was added to the solution. Then, the solution was stirred with a glass rod for 5 min, 20 ml of distilled water was added, and the obtained solution was centrifuged at 5000 rpm. The floating liquid on the centrifuge tube was separated and again, 5 ml of distilled water was added to the remaining specimen. The abovementioned process was replicated by adding 6.5 ml of perchloric acid and 20 ml of distilled water. The two extracts obtained were combined, and the perchloric acid in the mixture obtained interfered with the reaction between starch and anthrone, which must be evaporated by a hot water bath at 90 °C. The remaining solution was diluted with water to produce a concentration of 100 μg of starch

per liter of the extract. After this process, the solution was ready to be decomposed with anthrone reagent.

Measurement of metabolizable energy types:

Apparent Metabolizable Energy (AME):

Apparent Metabolizable Energy (AME) is sometimes also interpreted as classical metabolizable energy. It is the difference between the raw energy (measured by bomb calorimeter) of feedstuff consumed and the total energy excreted in feces and urine, which are excreted together in poultry (19).

Nitrogen-corrected Apparent Metabolizable Energy (AMEn):

Nitrogen-corrected Apparent Metabolizable Energy (AMEn) is the most commonly used estimate of metabolizable energy. It differs from AME corrected to retained nitrogen. This correction may be positive or negative based on the protein conservation status of the bird.

True Metabolizable Energy (TME):

True Metabolizable Energy (TME) was a measure used to estimate the metabolizable energy corrected for the metabolic energy of feces and the endogenous energy of urine. Metabolic energy is a part of faecal energy that doesn't originate from food and is caused by eroded intestinal mucosa, bile, and digestive fluids. Endogenous urine energy is also a part of urine energy that does not directly originate from food. The metabolizable energy obtained in this way is usually 5 to 10% greater than the apparent metabolizable energy.

Nitrogen-corrected True Metabolizable Energy (TMEn):

Nitrogen-corrected True Metabolizable Energy (TMEn) is True Metabolizable Energy (TME) corrected to zero nitrogen retention. In fact, the relationship between AMEn and AME is established between TMEn and TME. To determine TMEn values, in addition to calculating metabolic faecal energy and endogenous urine energy, the nitrogen retained in hungry chickens should also be calculated.

Equation 1: $AMEn = (Fi \times GEf) - (E \times GEe) / Fi$

Equation 2: $AMEn = ((Fi \times GEf) - (E \times GEe)) + (NR + K) / Fi$

Where, NR is calculated as follows: $NR=(Fi \times Nf)-(E \times Ne)$

Equation 3: $TME = \{((Fi \times GEf) - (E \times GEe)) + (FmE + UeE)\} / Fi$

Equation 4: $TMEn = ((Fi \times GEf)- (E \times GEe) - (NR \times K)) + ((FmE + UeE) + (NR0 \times K)/Fi$

Where, NR_0 is calculated as follows: $NR_0 =(Fi \times Nf)-(E \times Ne)$

Statistical and regression analyses:

In statistical models, regression is a statistical process to estimate the relationship between variables. Here, it estimates the relationships between the dependent variables (AME, AMEn, TME, and TMEn) and independent variables (crude protein, crude fat, crude fiber, ash, starch, glucose, and raw energy), and shows how the value of the dependent variable changes as each of the 2 independent variables changes. The coefficient of determination (R) is a statistical model that shows to what extent the total variation of ME is explained by chemical composition and the root-mean-square error (RMSE), which is equal to the standard deviation in the prediction, was used in the analyses.

Multiple linear regression and Gaussian process regression (GPR) models:

In many studies, GPR modeling is used to develop models providing accurate prediction of output variables. Comparison of actual and predicted output values may determine the performance of the prediction model based on the studied inputs. The proposed GPR model can predict TMEn appropriately in the validation data set that was not used during the training steps.

To evaluate the performance of prediction models, the R^2 value is calculated as a common measure to judge the "correctness" of the model considering its predictions, while the RMSE is usually used to indicate the "accuracy" of the model based on the test residual (error). Consequently, it is preferable to consider a combination of measures to determine or compare the overall performance of the prediction process. In TMEn modeling, R^2 and RMSE showed higher correctness and accuracy of the GPR model than the MLR model in the prediction of the concentration of chemical elements (31). Therefore, the present study proposes the GPR approach to predict the TMEn of rapeseed specimens for poultry considering the chemical composition of the feedstuff, including crude protein, crude fat, crude fiber, and ash. The developed GPR model produces relatively better values of TMEn in rapeseed specimens than those produced by conventional regression. The GPR model can improve the ability and capacity to accurately predict the energy content of diets to achieve optimal conditions in poultry nutrition.

RESULTS:

Chemical composition:

Table 2- The results of chemical analysis of rapeseed specimens in the raw form

Measure[1]	*Dry matter (%)*	*Crude protein (%)*	*Crude fat (%)*	*Crude fiber (%)*	*Ash (%)*	*Starch (%)*	*Glucose (%)*
Mean	*89.91*	*9.25*	*3.18*	*3.83*	*1.35*	*65.23*	*9.69*
Min	*87.95*	*7.4*	*2.36*	*3.19*	*1.03*	*54.80*	*8.88*

Max	*91.67*	*11.27*	*4.41*	*4.49*	*1.99*	*75.63*	*11.03*
CV	*0.69*	*7.68*	*15.70*	*7.47*	*16.67*	*8.36*	*5.21*

1. The average dry matter in all samples is 90% and the total number of experimental analyses performed on 30 rapeseed specimens in 4 independent replicates is 120 (30 specimens in each replicate).

Table 2 presents the results of the chemical analysis of raw rapeseed specimens. As seen in this table, the raw rapeseed specimens included average raw dry matter (89.91%), crude protein (9.25%), crude fat (3.18%), crude fiber (3.83%), ash (1.35%), starch (65.23%), and glucose (9.69%), and ash and dry matter had the highest (16.67%) and lowest (0.69%) coefficient of variation (CV), respectively.

In a study, the average crude protein and average crude fat were reported at 8.28% and 3.84%, respectively (36). In another experiment (13), it was reported that the protein and fat content of corn were 9.79% and 4.54%, respectively. Crude protein content in rapeseed may be the result of rapeseed production programs designed to increase its yield per hectare. The protein content of rapeseed can vary widely due to the type of cultivation by plant breeders. However, some other factors that may affect rapeseed protein content include rapeseed harvest season (14), soil fertility, crop management (especially nitrogen fertilizers), and weather conditions (8).

There was no relationship between the contents of crude protein and crude fat in the conventional hybrid cultivars of rapeseed. The commercial hybrid cultivar of rapeseed are developed to maximize the yield of rapeseed. The result was the random distribution of oil-containing genes in rapeseed. As the hybrid yield of rapeseed increased, the percentage of oil decreased. During the period from 1954 to 1982, the average yield of rapeseed in Illinois increased from 2.4 to 7.3 tons/ha (38), and the content of some elements was as follows: ash (0.3-3%), starch (64-75%), and crude fat (2.85-5.5%).

The effects of particle size on nutrient digestibility in pigs and broilers are well known (7, 40). Most of the rapeseed starch and protein were in its endosperm and they couldn't increased simultaneously. Most of the rapeseed oil is in the bud part and the increased endosperm affects the size of the origin. Therefore, the increased endosperm decreased the rapeseed oil content. The starch content in conventional hybrid cultivars of rapeseed (73.98%) was similar to the result reported by Soltwedel (2016).

There is little information to compare starch content. Rapeseed kernel cell wall components and structural carbohydrates (cellulose, hemicellulose, and pectin materials) can be produced as complex compounds with free fatty acids and minerals (i.e. phytate). Comparing this result with reports (26, 28) shows the lowest average crude protein and crude fat compared to those reported by NRC. The energy content in rapeseed is influenced by the content of nitrogen and rapeseed oil. The average crude protein and average crude fat (8.3% and 3.9%, respectively) were reported by NRC (24, 27 and 38). Douglas et al. (13) reported the protein and fat content of rapeseed at 9.7% and 4.49%, respectively. Low feed intake relative to maintenance requirements may alter the physiological status of birds, and small amounts of faeces can dramatically alter the endogenous losses/total exogenous losses ratio. These changes profoundly affect the accuracy of AME, AMEn, and TME as estimates of bioavailable energy (39).

There is a difference between rapeseed specimens in the chemical composition and metabolizable energy. The differences in the amount of dry matter can be attributed to the climatic conditions during harvesting and storage. It has been shown that there is a relationship between starch digestibility and metabolizable energy in cereal grains. Therefore, the presence of compounds such as non-starch polysaccharides (NSP), that limit the digestion of starch, can reduce the metabolizable energy in them. The amounts of starch and NSP in the seed are influenced by the climate and geography of the area (12). Therefore, the difference in weather conditions, agricultural management, and the variety of varieties used are effective factors in creating differences between specimens in the metabolizable energy. The difference between the rapeseed specimens in the metabolizable energy can be attributed to the difference in the crude fiber, crude protein, crude energy, and crude fat. Since the feedstuff energy is supplied by its organic matter part, any change in the amount of organic matter due to genetic and climatic factors causes a change in the metabolizable energy. Early harvesting of rapeseed before its maturity reduces its specific seed weight, starch content, and metabolizable energy (39).

Table 3- The results of the chemical analysis of rapeseed specimens in the digestible form

Measure[1]	*Dry matter (%)*	*Digestible nitrogen (%)*	*Digestible fat (%)*	*Digestible fiber (%)*	*Digestible ash (%)*
Mean	*84.5*	*55.79*	*83.21*	*66.01*	*64.6*
Min	*82.7*	*47.01*	*77.6*	*56.29*	*41.01*
Max	*86*	*67.19*	*88.78*	*74.01*	*87.6*
CV	*0.88*	*8.15*	*3.38*	*6.2*	*12.26*

1. All obtained chemical components were standardized based on the dry matter of the specimens. The average dry matter in all samples is 90% and the total number of experimental analyses performed on 30 rapeseed specimens in 4 independent replicates is 120 (30 specimens in each replicate).

Table 4- The results of the chemical analysis of rapeseed specimens in the retained form

Measure[1]	*Dry matter (%)*	*Retained nitrogen (%)*	*Retained fat (%)*	*Retained fiber (%)*	*Retained ash (%)*
Mean	*81.01*	*31.3*	*75.2*	*52.38*	*41.49*
Min	*79.1*	*18.11*	*66.5*	*40.19*	*24.2*
Max	*83.4*	*47.01*	*82.01*	*61*	*60.5*
CV	*1.22*	*18.72*	*4.21*	*8.43*	*17.53*

1. All obtained chemical components were standardized based on the dry matter of the specimens. The average dry matter in all samples is 90% and the total number of experimental analyses performed on 30 rapeseed specimens in 4 independent replicates is 120 (30 specimens in each replicate).

Table 4 shows the results of the chemical analysis of rapeseed specimens in the retained form as follows: average retained dry matter (81.01%), retained nitrogen (31.3%), retained fat (75.2%), retained fiber (52.38%), and retained ash (41.49%). The retained nitrogen and retained dry matter had the highest (18.72%) and lowest (1.22%) CV, respectively. In chickens, nitrogen is excreted in the form of uric acid. Per each gram of nitrogen excreted as uric acid, 256.8 kcal of body energy is lost and appears in the urine (excretion). However, those chickens storing protein lose less energy by excreting uric acid into the water. Therefore, the amount of ME may vary among different birds, due to differences in the amount of protein consumed and retained by the bird if they receive the same diet. The value of ME is independent of the conditions under which they are derived, it is too complicated to modify them to what they might be under standard conditions. The most widely used standard is that the birds are in nitrogen balance (where the amount of nitrogen reaches zero) (22).

Table 5- The results of metabolizable energy measurements of rapeseed specimens

Measure	*GE Kcal /kg*	*AME Kcal /kg*	*AMEn Kcal /kg*	*TME Kcal /kg*	*TMEn Kcal /kg*
Mean	*4365*	*3274*	*3209*	*3863*	*3771*
Min	*4150*	*2948*	*2892*	*3535*	*3451*
Max	*4490*	*3543*	*3478*	*4134*	*4041*
CV	*1.53*	*3.55*	*3.53*	*3.01*	*2.89*

GE: raw energy, AME: Apparent metabolizable energy, AMEn: Nitrogen-corrected Apparent Metabolizable Energy, TME: True Metabolizable Energy, TMEn: Nitrogen-corrected True Metabolizable Energy.

Table 5 shows the results of various metabolizable energy measurements of rapeseed specimens as follows: average raw energy (4365 kcal/kg and CV=1.53%), average AME (3274 kcal/kg and CV=3.55%), average AMEn (3209 kcal/kg and CV=3.53%), TME (3863 kcal/kg and CV=3.01%), and TMEn (3771 kcal/kg and CV=2.89%).

In the 1994 NRC report, the average AME was reported at 3764 kcal/kg. Earle (1977) reported the AME value of corn as 3815 kcal/kg. Another study reported the AME value of corn specimens as 3951 kcal/kg. These differences in the AME value can be related to the difference in the replacement methods used or uniformity in the evaluated specimens. Also, the difference among these studies can be due to the use of different corn hybrids (16,36).

A major advantage of applying a replacement method (i.e. AME) using a practical diet, is that the reference diet serves as a standard and is measured in each experiment, but in force-feeding methods introduced by Sibbald (27), the amount of food consumed is known precisely, and no diet is used as a base reference for estimations. One of its disadvantages is that the AME value of the food may differ from the composition of the reference diet. Some researchers (11,24,29) reported that the mono diet (feeding one type of food without mixing it with other diet components) is highly satisfactory for some grains, such as corn, but not for all other grains, such as wheat and barley.

Kanj Kato et al. (2011) reported the energy content of rapeseed in broilers from the age of one day to the end of the rearing period as follows: average AME (3839 kcal/kg), AMEn (3929 kcal/kg), TME (3754 kcal/kg), and TMEn (3812 kcal/kg). It can be inferred that the digestive system of the birds used in this case was developed and, accordingly, the ability to use nutrients has already been determined. In the NRC report (27, 28), the average AME value was 3764 kcal/kg higher than that in our study. Douglas et al. (1990) reported the amount of AME of yellow corn as 3815 kcal/kg (9). According to the Israeli convention, AME was 3950 kcal/kg. It used the ME value as determined by replacement methods. Differences among these studies could be due to the use of different corn hybrids (21). Wheat is the only satisfactory nutrition. However, testing a single ingredient for several foods is satisfactory, but not recommended, because many foods are unhealthy when fed alone (36).

Bourdillon et al. (2017) reported that the AMEn content of the experimental diet was always lower when measured in younger birds than in older ones. The value of AMEn in young birds was lower than in old ones, which has been previously reported in many experiments (18,10, 19, 29). Regarding the use of corn in the diet, compared to the results of old roosters, the changes in the amount of AME in young chickens seem to be slightly higher (9.4% vs. 7.5%). This agrees with the study by Bourdillon et al. (1990). Comparing

the results with the NRC report (27), where AME was 3889 kcal/kg for corn, and the average TMEn was 4001 kcal/kg, shows a difference between them.

Analysis of variance:

Table 6- The results of the variance analysis of rapeseed specimens in the raw form

Country	*Dry matter (%)*	*Crude protein (%)*	*Crude fat (%)*	*Crude fiber (%)*	*Ash (%)*	*Starch (%)*	*Glucose (%)*	*Raw energy (%)*
Iran	*90.34^{a}*	*8.74^{b}*	*3.20*	*3.86^{a}*	*1.30^{ab}*	*64.03^{bc}*	*9.61*	*4386^{a}*
Ukraine	*89.61^{b}*	*8.85^{b}*	*3.17*	*3.97^{a}*	*1.29^{b}*	*66.84^{ab}*	*9.83*	*4326^{b}*
Canada	*89.79^{b}*	*9.08^{b}*	*3.15*	*3.68^{b}*	*1.45^{a}*	*62.81^{c}*	*9.74*	*4370^{a}*
China	*89.93^{ab}*	*9.61^{a}*	*3.05*	*3.75^{ab}*	*1.31^{ab}*	*68.19^{a}*	*9.46*	*4392^{a}*
SE	*0.05*	*0.05*	*0.04*	*0.03*	*0.02*	*0.41*	*0.04*	*6.41*
P-Value	*0.01 ≤*	*0.01 ≤*	*0.43*	*0.01 ≤*	*0.02*	*0.01 ≤*	*0.06*	*0.01 ≤*

Table 6 presents the results of the variance analysis (dry matter, crude protein, crude fat, crude fiber, ash, starch, glucose, and crude energy) for the tested rapeseed. In this table, Iranian rapeseed specimens had the highest average dry matter (90.34%), and Ukrainian and Canadian specimens had the lowest average dry matter (89.61 and 89.79). Also, Chinese specimens had the highest crude protein content (9.6%), and the Iranian, Ukrainian, and Canadian specimens had the lowest average crude protein content (8.85%, 8.74%, and 9.08%, respectively). For crude fat, none of the specimens were significant at Sig.=0.01. The Iranian, and Ukrainian specimens had the highest average raw fiber and the Canadian specimens had the lowest one, and the specimens were significant. The Canadian specimen had the highest average ash content (1.45) and the Ukrainian specimens had the lowest one (1.29). The Chinese specimens had the highest average starch content (62.81) and the Canadian specimens had the lowest one. None of the specimens was significant for glucose. Regarding crude energy, the Iranian, Canadian, and Chinese specimens had the highest values (4386, 4370, and 4392) and the Ukraine specimens had the lowest value, and the specimens were significant.

Correlation coefficient:

Table 7- Correlation coefficients between chemical compounds for specimens in the raw form

	Dry matter (%)	*Crude protein (%)*	*Crude fat (%)*	*Crude fiber (%)*	*Ash (%)*	*Starch (%)*	*Glucose (%)*	*Raw energy (Kcal/kg)*
Crude protein (%)								
P-Value	*NS*							
Crude fat (%)	*0.33*	*0.05*						
P-Value	*	*NS*						
Crude fiber (%)	*-0.006*	*-0.05*	*-0.11*					
P-Value	*NS*	*NS*	*NS*					
Ash (%)	*-0.26*	*0.12*	*-0.08*	*-0.54*				
P-Value	*	*NS*	*NS*	*				
Starch (%)	*-0.12*	*-0.03*	*-0.26*	*-0.08*	*0.10*			
P-Value	*NS*	*NS*	*	*NS*	*NS*			
Glucose (%)	*-0.26*	*0.09*	*-0.24*	*0.09*	*0.17*	*0.22*		
P-Value	*	*NS*	*	*NS*	**	**		
Raw energy (Kcal/kg)	*0.41*	*0.35*	*0.01*	*-0.27*	*0.07*	*-0.1*	*0.08*	
P-Value	*	*	*NS*	*	*NS*	*NS*	*NS*	

NS: non-significant, *: significant at P>0.01, **: significant at P>0.05.

Table 7 shows the correlation matrix between the chemical compounds of rapeseed specimens using the specimen analysis data. The highest positive correlation (0.42) was observed between dry matter and raw energy (P<0.01), and the highest negative correlation (-0.54) was observed between raw fiber and ash (P>0.01).

Table 8- Correlation coefficients between chemical compounds for rapeseed specimens in the digestible and retained forms

	Dry matter (%)	*Digestible nitrogen (%)*	*Digestible fat (%)*	*Digestible fiber (%)*	*Digestible ash (%)*	*Dry matter (%)*	*Retained nitrogen (%)*	*Retained fat (%)*	*Retained fiber (%)*

Digestible nitrogen	*-0.06*								
P-Value	*NS*								
Digestible fat	*0.14*	*0.06*							
P-Value	*NS*	*NS*							
Digestible fiber	*0.20*	*-0.01*	*0.45*						
P-Value	**	*NS*	*						
Digestible ash	*-0.22*	*-0.19*	*-0.1*	*-0.07*					
P-Value	*	**	*NS*	*NS*					
Retained Dry matter	*0.95*	*-0.02*	*0.29*	*0.37*	*-0.29*				
P-Value	*	*NS*	*	*	*				
Retained nitrogen	*-0.08*	*0.95*	*0.11*	*-0.01*	*-0.14*	*-0.05*			
P-Value	*NS*	*	*NS*	*NS*	*NS*	*NS*			
Retained fat	*0.37*	*0.11*	*0.87*	*0.55*	*-0.21*	*0.54*	*0.13*		
P-Value	*	*NS*	*	*	**	*	*NS*		
Retained fiber	*0.2*	*-0.01*	*0.51*	*0.96*	*-0.01*	*0.37*	*-0.02*	*0.6*	
P-Value	**	*NS*	*	*	*NS*	*	*NS*	*	
Retained ash	*-0.43*	*-0.1*	*-0.19*	*-0.21*	*0.89*	*0.52*	*-0.04*	*-0.3*	*-0.18*
P-Value	*	*NS*	**	**	*	*	*NS*	*	**

NS: non-significant, *: significant at P>0.01, **: significant at P>0.05.

Table 8 presents the correlation coefficients between digestible and retained chemical compositions of rapeseed specimens. The highest positive correlation (0.96) was observed between retained fiber and digestible fiber (P<0.01). and the highest negative correlation (-0.52) was observed between retained ash and retained dry matter (P < 0.01). Zhao et al. (2008) reported that GE was positively correlated with CF, ADF, NDF, CP, and EE. However, THE correlation between ME and GE was negative, which is inconsistent with the results of (20). The chemical compounds significantly correlated to ME, as well as NDF, CF, and ADF. ME and NDF had the highest correlation with these 3 fibers, showing that the effect of CF and ADF on the ME content of calibration specimens can be explained by NDF content. Therefore, the results showed that the ME content of soybean calibration specimens in adult ducks may largely depend on NDF and GE. Similar observations have been reported about the ME content of rapeseed in broiler chickens (17). There was a strong and positive correlation between Crude fat and GE (r<0.85). Starch and soluble carbohydrates had a strong negative correlation with GE (-0.66 and -0.81, respectively). There was a strong and negative correlation between Crude fat and starch. Crude fiber and protein had a strong correlation (36). Strong correlations changed the contribution of variables to the overall R^2 model depending on the presence or absence of other variables. Whenever EE or GE were included in the same model, each accounted for 70% of the variation in DE. As a result, there is a strong correlation (r=0.96) between EE and GE, including EE in the model with GE, which was part of R^2 for EE. The strong and positive correlation between GE and ME with fat and the negative correlation between GE and starch in the rapeseed specimen evaluation were used in developing AMEn prediction equations.

The correlation between the variables in the study is completely different from what was reported about the hybrid regimes. Compared to the results, (23, 33) reported strong and negative correlations between DE and ash (-0.64 and -0.65) but a positive correlation between DE and starch (0.79 and 0.49). These studies reported poor correlations between DE and EE (0.12 to 0.3) or GE (0.28 to 0.3). Since the predicted variables with DE, the relationships between predicted variables in feeds consisting of various ingredients and mono diet such as rapeseed, the relative weight of predicting variables, and the important combination of variables in the DE prediction model based on hybrid regimens may be different, it is not appropriate to use models based on hybrid diets to predict DE for rapeseed-based diets.

Regression equations:

The multiple linear regression model (MLR) obtained in the rapeseed data set was as follows:

DM= 3896+56.3CP+30.1EE-122.5CF-178.1Ash)kcal/kg (*TMEn of sample*

R^2=0.22 , RMSE = 104.7 kcal/kg of DM

The values of input variables were considered in the percentage of dry matter. All factors estimated, except for crude fat, were significantly different from zero ($p < 0.05$), while crude fat tends to be significant ($p = 0.1$). The R^2 value shows that only 23% of the variations in responses are explained by the developed model. When faced with unpredicted data, the average prediction error is 104.8 kcal/kg (found with the RMSE value).

Table 9- Fitted regression equations for the types of estimated energy (AME, AMEn, TME, and TMEn) in kcal per kg of dry matter for the specimens

Equation 1	*RMSE = 82.64 R^2 = 55.73*	*AME=-1980+ 31.6 CP+35.6 EE-52,5 CF-146.2 Ash-1.01 STA+21.9 GLU+0.972 GE*
Equation 2	*RMSE = 82.65 R^2 = 55.33*	*AME=-1551+30.6 CP+38.6 EE-105.6 Ash+1.039 GE*
Equation 3	*RMSE = 103.3 R^2 = 29.36*	*AME= 3487+ 63.1 CP-159.01 CF-214.7 Ash- 3.68 STA+39.3 GLU*
Equation 4	*RMSE = 81.9 R^2 =54.25*	*AMEn=-1079+19.7 CP+34.5 EE- 52.5 CF- 145.8 Ash-1.12 STA+ 21 GLU+0.981 GE*
Equation 5	*RMSE = 82.52 R2 = 51.8*	*AMEn= -1668+36.3 EE-100.2 Ash+1.12 GE*
Equation 6	*RMSE = 105.2 R2 = 21.8*	*AMEn = 3532+55.6 CP-137 CF-194.7 Ash*
Equation 7	*RMSE = 83.03 R2 = 55.47*	*TME=-526+31.4 CP- 35.4 EE-52CF-145.5 Ash-1.05 STA+22 GLU+0.975 GE*
Equation 8	*RMSE = 83.13 R2 = 54.18*	*TME = -976+30.4 CP+ 38.2 EE- 105.1 Ash+1.05 GE*
Equation 9	*RMSE = 103.71 R2 = 29.29*	*TME= 4094+62.9 CP+159.7 CF- 214 Ash- 3.76 STA+39.5 GLU*
Equation 10	*RMSE = 82.25 R2 = 53.99*	*TMEn=-530+ 19.4 CP+34.2 EE-52 CF-145 Ash-1.09 STA+ 21 GLU+ 0.983 GE*
Equation 11	*RMSE = 83.09 R2 = 51.46*	*TMEn =-1089+34.03EE-98.7 Ash+1.02 GE*
Equation 12	*RMSE =104.5 R^2 =21.47*	*TMEn =4102+56.3CP+136.6CF-194.11 Ash*

R^2: coefficient of determination, RMSE: root-mean-square error. In these equations, crude protein (CP), crude fat (EE), crude fiber (CF), ash (Ash), starch (STA), and glucose (GLU) are included in the percentage of the dry matter of the specimens and crude energy (GE) in Kcal/ kg of dry matter.

As seen in Table 9, to predict the value of AME using equations 1, 2, and 3, the R^2 values were estimated as 55.7%, 55.3%, and 29.3%, respectively. It was found that crude protein, crude fat, ash, and raw energy could have a stronger relationship with AME than other variables. By removing the crude fiber, starch, and glucose parameters from Eq.1, the R^2 value was estimated as 55.7 and the RSME value as 82.6 for Eq. 2, showing that these parameters have a significant role in the equation. Also, in Eq. 3, with the presence of crude protein, crude fat, crude fiber, ash, starch, and glucose, the R^2 value decreased and the RMSE value increased by 103.3, indicating the effect of all parameters of Eq.2. Eq. 3 is the simplest equation that can predict AME in rapeseed specimens, although the R^2 decreased by about 25% and the RMSE increased. It is a simply applicable equation. Predicting AMEn using equations 4, 5, and 6 presented the R^2 values of 54.2%, 51.8%, and 21.8%, respectively, and it was found that crude fat, ash, and crude energy could have a stronger relationship with AME than other variables. By removing the crude protein, crude fiber, starch, and glucose parameters from Eq.4, the R^2 value was estimated as 51.8% and the RMSE value as 82.52 for Eq. 5, showing that the removed parameters have no significant role in the equation. With the presence of crude protein, raw fiber, and ash, the R^2 value was estimated as 21.81 and the RMSE value as 105.2 for Eq.6. Eq. 6 is the simplest equation that can predict AMEn in rapeseed specimens. Although the R^2 value decreased by about 30% and the RMSE value increased, it is a simply applicable equation.

To predict TME using equations 7, 8, and 9, the R^2 value was estimated as 53.99%, 54.1%, and 29.2%, respectively. The amount of crude protein, crude fat, ash, and crude energy could have a stronger relationship with TME than other variables. By removing the crude fiber, starch, and glucose parameters from Eq.7, the R^2 value was estimated as 54.18 and the RMSE value as 83.13 for Eq.8, indicating the significant role of these parameters in the equation. With the parameters of crude protein,

crude fiber, ash, starch, and glucose in Eq.9, the R^2 value decreased and the RMSE value increased by 103.71.

To predict TMEn using equations 10, 11, and 12, the R^2 value was estimated as 55.4%, 54.1%, and 29.2%, respectively. The estimates indicate that crude fat, ash, and crude energy can have a stronger relationship with TMEn than other variables. This greatly contributes to the expression of TMEn. By removing the crude protein, crude fiber, starch, and glucose parameters from Eq.10, the R^2 value was estimated as 51.46 and the RMSE value as 83.09 for Eq. 11, indicating that these parameters had no significant role in the equation. With the parameters of crude protein, crude fat, crude fiber, and ash, the R^2 value was estimated as 21.47 and the RMSE value as 104.5 for Eq.12, indicating that the parameters of Eq. 11 are significant. However, this equation is more complicated and has less practical appeal due to the presence of the GE variable. Eq. 12 is the simplest equation that can predict TMEn in rapeseed specimens. Although the R^2 value decreased by about 30% and the RMSE value increased, it is a simply applicable equation.

The low accuracy (according to the R2 value) and the relatively high error of the applied equations used for predicting rapeseed energy in the present study and similar ones (13, 42) are due to the gathering of the assessed specimens from a wide range of specimens in factories with different imported sources. Similar results were observed for monogastric animals when different specimens of rapeseed were collected from different regions and used for biological analyses and statistical modeling (30, 36).

Correlations between analyses observed for rapeseed resources were applied to all rapeseed diets. Therefore, in such a situation, the correlation between the predicting variables causes no problem in prediction, unless they are extracted (34). These correlations between the predicted variables led to several competing models with similar ability to predict. In general, models with 4 or 5 variables have more similar R^2 and RMSE than other models. The difference between these two model groups in R^2 and RMSE was small. Therefore, further evaluation was done on 4-variable models, including possible interactions between the variables.

Gaussian Process Regression (GPR) model:

GPR modeling is used to develop models providing accurate prediction of output variables. Comparison of actual and predicted output values may determine the performance of the prediction model based on the studied inputs. The proposed GPR model can predict TMEn appropriately in the validation data set that was not used during the training steps.

Table 10- Data used in the predictive modeling process

Model input	*N*	*Min*	*Max*	*Mean*	*SD*
Crude protein % of Dry matter	*120*	*7.40*	*11.28*	*9.05*	*0.702*
Crude fat % of Dry matter	*120*	*2.36*	*4.40*	*3.18*	*0.508*
Crude fiber % of Dry matter	*120*	*3.19*	*4.49*	*3.83*	*0.287*
Ash % of Dry matter	*120*	*1.03*	*1.99*	*8.43*	*0.233*
Model output TMEn kcal/kg	*120*	*3461.49*	*4051.51*	*3781.33*	*117.53*

30 rapeseed specimens (Canada (n=9), China (n=5), Iran (n=7), and Ukraine (n= 9)) were presented. All measures were studied with 4 replicates for each rapeseed specimen. See the completed data set in the appendices.

Descriptive statistics of the data used in the predictive modeling process are listed in Table 10. In this table, the total number of rapeseed specimens was 120 and the model inputs (crude protein, crude fat, crude fiber, and ash) are listed in the percentage of the dry matter, and the model output (TMEn) in kcal/kg. The model inputs were as follows: crude protein (9.05%), crude fat (3.18%), crude fiber (3.83%), ash (8.43%), and the model output, i.e. average TMEn was 3781.4 kcal/kg. As a result, the present study proposes the GPR approach to predict the TMEn of rapeseed specimens for poultry considering the chemical composition of the feedstuff, including crude protein, crude fat, crude fiber, and ash. The developed GPR model produces relatively better values of TMEn in rapeseed specimens than those produced by conventional regression. The GPR model can improve the ability and capacity to accurately predict the energy content of diets to achieve optimal conditions in poultry nutrition.

CONCLUSION:

The present research results showed the relative difference between chemical compositions and metabolizable energy calculated for rapeseed specimens collected from different provinces of Iran with what was mentioned in conventional poultry sources. Therefore, in practical conditions in the poultry industry and animal feed factories, it is necessary to perform new studies to use more local models. The chemical composition and energy content calculated for rapeseed specimens were significantly different from what was mentioned in the poultry food standards tables, which can be due to the way of storage, weather conditions, seed type, product management, and harvesting season in different places. It was found that TMEn was positively correlated to

crude protein and crude fat, while there was a negative correlation between TMEn and ash. The highest amount of TMEn was related to the specimens with the highest amount of crude fat and the lowest amount of ash. The prediction equations obtained in this research can be used to estimate the energy of different rapeseed specimens in animal feed factories or poultry farms. This study showed that specimens from different origins differ in chemical composition and GE, but no difference was observed in TMEn content. The proposed GPR procedure successfully predicted the TMEn of rapeseed specimens for poultry according to their chemical compositions (CP, EE, CF, and Ash). GPR can improve the ability and capacity to accurately predict the energy content of feedstuffs to achieve optimal diets for poultry nutrition.

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