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Determination of Chemical Composition and Apparent Metabolizable Energy Corrected for Nitrogen (AMEn) Content of Amaranth Grain with and without Enzyme in Adult Leghorn Roosters by Regression Method

Research Article

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ABSTRACT

To determine chemical composition as well as apparent metabolizable energy corrected to zero nitrogen balance (AMEn) estimates of Amaranth grain in adult roosters by regression method, firstly, a specie of *Amaranthus hybridus chorostachys* were cultivated in a farm pilot at agriculture research station (Khalat Poushan-Tabriz- East Azarbaijan- Iran). After harvesting, amaranth grain was sifted and milled. Finally, the chemical composition of amaranth grain produced in the Advanced Animal Nutrition Laboratory was analyzed. Adult roosters 96-week-old Leghorn in individual metabolic cages for nine days used to determine AMEn content by the regression method. The basal diet was based on corn-soybean with 3200 kcal/kg metabolizable energy and 12% protein. Two experiments with raw amaranth grain (RAG) and processing amaranth grain (PAG), started separately using 40 leghorn roosters in the form of completely random by factorial method (2×5), that each experiment consisted of two levels of Rovabio multi-enzyme (0 and 0.055%) and five amaranth grain levels (0, 15, 30, 45, 60% of replacement or embedment of energizing part of the diet). Each experiment included four replicates that included one bird, it was subjected to biological experiments in metabolic cages by total excreta collection method. The obtained results of regression equations in adult roosters showed that AMEn content for raw amaranth without enzyme (RAG^{-e}), raw amaranth with an enzyme (RAG^{+e}), processed amaranth without enzyme (PAG^{-e}), and processed amaranth with an enzyme (PAG^{+e}) were 3250.24, 3433.18, 3242.18 and 3438.09 kcal/kg, respectively. The total results of this study showed that according to amaranth's adaptable and nutritious agricultural features, it can have a good potential in supplementation of poultry diets with enzymes for improving the energy and nutrition quality.

KEY WORDS adult roosters, amaranth grain, enzyme, metabolic cages, metabolizable energy, poultry diets.

INTRODUCTION

According to the Food Agriculture Organization (FAO) and the World Health Organization (WHO), Amaranth after the egg and upper than cow's milk, is the second most valuable substance in the world in the protein value chart (Cai *et al.* 2003). Amaranth is the most important plant from Pseudocereals (FAO/WHO/UNU, 1985).

Pseudocereals are plants that produce fruits or seeds which are used and consumed as grains, though botanically pseudocereals are neither grasses nor true cereal grains. Pseudocereals are typically high in protein and other nutrients, gluten-free, and are considered whole grains. Many so-called "ancient grains" are pseudocereals (Gordon, 2006). Today, the introduction and development of forgotten plants such as pseudocereals have been able to help

agricultural problems and improve human's nutritional along with genetically modified plants under the name of new crops (Rastogi and Shukla, 2013).

Estimates indicate that the need for cereals, which is one of the most important consumables in poultry nutrition, on a global scale to meet the demand for cereals by 2025 would need an increase in wheat production of 44%, rice production of 43%, and corn production of 56% compared to those of in 2000 (Khush, 1999; Rosegrant *et al*. 2001). Due to the high protein quality and amino acid balance of Amaranth, which is similar to animal protein sources, as well as the high levels of other nutrients in Amaranth, it has been able to play a valuable role in complementing and replacing nutritional programs in the fight against malnutrition and possible deficiencies of nutrients, especially from cereal sources in dietary foods. According to reports, lysine, methionine and arginine content in Amaranth are 2 to 3 times higher than common legumes (peas, beans, soya, etc.) (Cai *et al*. 2003).

Today Amaranth has become a world-class product of high potential and multipurpose use (Rastogi and Shukla, 2013).

The respiratory system in the category of C_4 plants belongs to the genus amaranthus (which includes 75 species) and the family amaranthcea (Mozaffarian, 2013). In Iran, 11 species of amaranth are grown and other common names in Persian besides "Taj Khoroos" are "Baroothak" and "Avisi" (Mozaffarian, 1996; Mozaffarian, 2013).

Considering the nutritional profile of this research on Amaranth and the classification of the Amaranth according to nutritional division, we can report that there are two major types of Amaranth (Cai *et al*. 2003). They are the Grain-like Amaranth producers: *Amaranthus cruentus*, *Amaranthus caudatus*, *Amaranthus hybridus*, *Amaranthus hypochondriacus,* and the Vegetable-like Amaranth: *Amaranthus tricolor*, *Amaranthus dubius*, *Amaranthus lividus* (Cai *et al*. 2003).

Amaranth hybridus is known as "Bari" in the world. At several points in the world, there are South America, Africa, India, China and the United States (He *et al.* 2002). Amaranth grains are small and sticky. Squalene values in the hybrid species (7.3%) were reported more than other species (Williams and Brenner, 1995; Budin *et al.* 1996).

Amaranth is a world-class herbaceous plant with a higher vegetative and resilient strength than any grain that can be obtained cheaper, and there is a potential for the entry of this plant and its products to nutritional programs (Rastogi and Shukla, 2013). Considering that energy is an important part of the poultry diet and accounts for about 40% of the cost of production, it is important to determine the nutritional energy of the food to properly regulate the poultry diet (Shivazad and Seidavi, 2006).

The purpose of this study was to focus on the nutritional potential of Amaranth by determining the chemical composition and numerous nutritional and biological tests on poultry nutrition (metabolic and adult rooster) to provide an accurate assessment of the nutritional value of amaranth.

MATERIALS AND METHODS

Planting and production of amaranth

To provide the raw material in this research, which is an herbicide and is an amaranth seed, amaranth farming was carried out in Khalat-Poushan research center (Tabriz, Azarbayjan). The species being studied was *A. hybridus*. Herbarium specimens of cultivated plants were sent to Iran Botanical Garden (National Botanical Garden of Iran) and approved. In the year 2016, amaranth cultivation was carried out in June and harvesting was in September. After harvest, the prepared samples were milled. Fifty percent of the product was crude (raw amaranth grain (RAG)) and fifty percent of the product was processed under autoclave heat treatment (humidity of 120 ˚C for 5 minutes) (processed amaranth grain (PAG)), that was the raw amaranth processed (RAP). The products were packaged before transferring them for biological and nutritional tests.

In addition to thermal processing, in 50 % of treatments, both in the case of grain and in processing enzymatic treatment was carried out of experimental diets (e^+). For regression testing, a multi-enzyme was used for enzyme-containing treatments. Rovabio is a multienzyme complex (commercial) and that it is used to increase the digestibility of cereals.

Each gram of the multi-enzyme, Rovabio, contains 22000 viscose units of Xylanase enzymes, 2000 units of the enzyme beta-glucanase, as well as other enzymes such as cellulase, pectinase, protease, beta-mannosidase. The reason for using this multi-enzyme Rovabio was to reduce the potential antinutrient effects of Amaranth grain, which is recommended in poultry nutrition as an additive. All samples were transferred to the Advanced Animal Nutrition Laboratory in Tabriz University for analysis and determination composition.

Proximate analysis

In the first stage of the experiments, the chemical and mineral composition of amaranth grain were analyzed according to the Institute of Standards and Industrial Research of Iran (ISIRI, 2003) (Table 1). The nutritional value analysis includes the determination of dry matter according to AOAC (1990) crude protein by Kjeldahl analysis (Kjeltec Analysis Foss 2300 Tecator) according to the reference method of ISIRI 19052, raw fats by Suk according to the method of ISIRI 415, Velp device, ash in electric furnace at

550 ˚C according to the reference method of ISIRI 103 and crude fiber with the Foss Tecator device according to the reference method of ISIRI 3961. Calcium was analyzed using Atomic absorption Shematzo spectroscopy according to the reference method of ISIRI 9266 and phosphorus by the spectrophotometric method (Apel, AA-6300) according to the reference method of ISIRI 513. The gross energy was calculated using a calorimetric bomber Par in the Advanced Animal Nutrition Laboratory in the Faculty of Animal Science, Faculty of the Agriculture University of Tabriz-Iran. Determination of the fatty acids profiles using HPLC QP2010SE GC-MS model based on Institute of Standards and Industrial Research of Iran (ISIRI) 13126-2 was implemented in the ARL (Afshin Rahimi Lab) located in Rasht, Guilan, Iran.

Birds and management

A total of 40 adult roosters (98 wk) were randomly assigned to each treatment. According to the protocol Bourdillon *et al*. (1990), the adult roosters were studied for 9 days (four days of adaptation to assay diets, one day of fasting, three days of assay diet, and one day of fasting). Due to the thermal processing of the sample as well as the use of the enzyme, the determination method (AMEn) at different levels of the substitution was done by a single regression method on adult Leghorn roosters.

According to McNab and Boorman (2002), the reason for using adult roosters was used due to their constant feed intake, low growth rate, higher feed intake than other birds, rapid adjustment to dietary changes, and long maintenance period, results are reported with less variance.

Additional reasons for using this bird are consistency, the strength of the digestive system, and the low maintenance requirements. In the regression method, we obtain equations that can be used to predict the amount of energy at each level of the use of the test sample in the experimental diet.

The location of these studies was in the adult metabolism laboratory hall at Khalat-Poushan Research Station, Faculty of Agriculture, Tabriz University. In this laboratory, individual cages of 40 × 45 × 40 cm in size were provided with a separate dish and a container for drinking water. The temperature of the metabolism salon of adult roosters was 26 ˚C and *ad libitum* water consumption, but an experimental diet of 100 g daily was provided for each bird to provide maintenance requirements.

Each experiment had 20 birds (five treatments, four replicates of one bird). Two separate assays, once on RAG and once again on PAG to determine AMEn content by regression assay were conducted in a completely randomized design with a factorial arrangement of (2×5) containing two levels of Rovabio enzymes (0 ($^{-e}$) and 0.055 ($^{+e}$) %) and in five different levels of experimental (0, 15, 30, 45 and 60% amaranth) and the first treatment was the basal diet and the other treatments included amaranth. Each treatment four replicates for adult Leghorn roosters contains a bird in each of the metabolic cages that were performed by the total excreta collection method. The order of using amaranth and enzyme in experimental diets was as follows in Tables 2 and 3.

A basal diet was formulated based on linear programming using UFFDA software (Table 4).

Table 1 Composition of amaranth grain (A. hybridus) and comparison of some corn and wheat composition in NRC (1994)

Item	Unit	A. hybridus (Test results)	Wheat (NRC, 1994)	Corn (NRC, 1994)
Dry matter	Weight percent	90.4	89	89
Crude protein	Weight percent	16.8	10.2	8.8
Crude fat	Weight percent	5.2	1.8	3.8
Crude fiber	Weight percent	10.1	2.4	2.2
Ash	Weight percent	5.6	-	1.5
Calcium	Weight percent	0.25	0.05	0.02
Phosphorous	Weight percent	0.56	0.31	0.28
Iron	mg/kg	39.4	-	-
Zinc	mg/kg	15.1	28	10
Copper	mg/kg	1.6	-	3
Manganese	mg/kg	34.8	24	7
Gross energy	kcal/kg	3749		
Fatty acids				
Palmitic acid (C16:0)	Percent of total fatty acids	7.66	-	-
Oleic acid (C18:1, n-9)	Percent of total fatty acids	15.22	-	-
Linoleic acid (C18:2, n-6)	Percent of total fatty acids	34.79	0	2.2
γ-linolenic acid (C18:3, n-6)	Percent of total fatty acids	0.30	-	-
α-linolenic acid (C18:3, n-3)	Percent of total fatty acids	0.37	-	-

Table 2 Design of first experiment treatments

First experiment (raw amaranth grain (RAG))			
Number of treatment	**Level of amaranth (%)**	**Enzyme without ($^{-e}$) and with ($^{+e}$)**	**Summarized**
1 (basal diet)	0	-	% 0 RAG^{-e}
2	15	-	% 15 RAG^{-e}
3	30	-	% 30 RAG^{-e}
4	45	-	% 45 RAG^{-e}
5	60	-	% 60 RAG^{-e}
6	0	+	% 0 RAG^{+e}
7	15	+	% 15 RAG^{+e}
8	30	+	% 30 RAG^{+e}
9	45	+	% 45 RAG^{+e}
10	60	+	% 60 RAG^{+e}

Table 3 Design of second experiment treatments

Second experiment (processed amaranth grain (PAG))			
Number of treatments	**Level of amaranth (%)**	**Enzyme without ($^{-e}$) and with ($^{+e}$)**	**Summarized**
1 (basal diet)	0	-	% 0 PAG^{-e}
2	15	-	% 15 PAG^{-e}
3	30	-	% 30 PAG^{-e}
4	45	-	% 45 PAG^{-e}
5	60	-	% 60 PAG^{-e}
6	0	+	% 0 PAG^{+e}
7	15	+	% 15 PAG^{+e}
8	30	+	% 30 PAG^{+e}
9	45	+	% 45 PAG^{+e}
10	60	+	% 60 PAG^{+e}

Table 4 Ingredient composition basal diet for maintenance requirements for adult roosters Leghorn

Ingredients (%)	**Amounts**
Corn	87.51
Soybean meal (44%)	10.69
Dicalcium phosphate	1.59
Oyster shell	1.30
Salt	0.36
Vitamin and mineral premix[1]	0.50
DL-methionine	0.05
Calculated analysis	
AME_n (kcal/kg)	3200
Crude protein (%)	12.00
Calcium (%)	0.85
Available phosphorus (%)	0.42

[1] Vitamin and mineral premix include per kilogram of diet: vitamin A: 7.2 g; vitamin D_3: 0.6 g; vitamin E: 14.4 g; Thiamin: 0.72 g; Riboflavin: 3.3 g; Nicotinic acid: 4 g; Pyridoxine: 1.2 g; Ciano Cobalamin: 0.6 g; Menadione: 1.6 g; Folic acid: 0.5 g; Choline chloride: 400 mg; Manganese oxide: 64 g; Iron: 44 g; Sulphate copper: 16 g; Iodine: 0.64 g and Selenium: 80 mg.

The basal diet was formulated only on the of the maintenance requirements (no growth and no production) of the adult rooster. As mentioned, according to protocol Bourdillon *et al*. (1990), the experimental diet and each of the prepared diets was first prepared for four days for adaptation *ad libitum*, followed by 24 hours of starvation, and then by placing special trays for collecting feces in a 3-day dietary basis with a daily intake of 100 grams.

Upon completion of this course, the animals were hungry for 24 hours to empty the digestive system so that the gastrointestinal tract was completely evacuated, but the accumulation of feces continued during these 24 hours.

Test ingredients and experimental diets

Chemical analysis of experimental diets and excreta samples of biological assay

Experimental rations and excreta collected at the end of the experiment and samples taken in biological experiments were transferred to the Advanced Animal Nutrition Laboratory. The specimens were kept at an oven temperature of 70 ˚C for 72 hours until the wastes dried. After evacuating from the oven and cooling in the refrigerator, samples were transferred to the lab for 24 hours to exchange moisture and then they were milled after weighing.

The samples were stored in plastic containers until the chemical decomposition. Chemical analysis of experimental diets and waste samples included determination of dry matter according to AOAC (1990), crude protein by Kjeldahl analysis (Kjeltec Analysis Foss 2300 Tecator) according to the reference method of ISIRI 19052, raw fats by Suk according to the reference method of ISIRI 415, Velp, raw ash in an electric furnace at 550 ˚C according to the reference method of ISIRI 103, crude fiber using atomic absorption spectrometry (Foss Tecator) according to the reference method of ISIRI 3961, atomic absorption spectrometry for calcium according to the method of ISIRI 9266 and phosphorus was also analyzed using (Apel, AA-6300) spectrometry according to the reference method of ISIRI 513. The amount of gross energy was measured using the spectrophotometric method using the calorimeter bomber par.

Calculations and statistics

In order to calculate the AME and AMEn in experimental diets, the formula presented by Sibbald and Wolynetz (1989) and Sibbald (1989) was used.

$$AME = \{[(F_i \times GE_f) - (E \times GE_e)] \div F_i\}$$
$$AMEn = \{[(F_i \times GE_f) - (E \times GE_e) - (NR \times K)] \div F_i\}$$

Where:
AME: apparent metabolizable energy (kcal/kg).
AMEn: apparent metabolizable energy corrected for nitrogen (kcal/kg).
F_i: feed intake.
E: excreta (g).
GE_f: gross energy feed intake (kcal/kg).
GE_e: gross energy excreta (kcal/kg).
$NR = (Fi \times N_f) - (E \times N_e)$.
NR: nitrogen retention.
Ne: percent of nitrogen excreta.
Nf: percent of nitrogen feed intake (feed nitrogen) (%).
K: 8.22 kcal/g nitrogen retained (nitrogen retention corrected coefficient).

Then, to calculate the apparent metabolizable energy of each amaranth sample in experimental treatments and to determine the prediction equations at different levels of replacement in the diet using the regression method, the formulas, and reports proposed in Cao and Adeola (2016) were used as follows:

$$C_{ti} = \{C_{bd} + [(C_{ad} - C_{bd}) \div P_{ti}]\}$$

Where:
C_{ti}: AMEn of test ingredient.
C_{bd}: AMEn of basal diet.
C_{ad}: AMEn of assay diet.
P_{ti}: amaranth replacement percentage**.**

All data related to the parameters were collected and sorted by Excel 2013 software. Before analyzing data, residual normality test and homogeneity of variance were performed. Data analysis was performed using "R" statistical software. Data were analyzed in a completely randomized design with factorial experiment. To compare the mean of treatments, the Duncan test was used. Regression analysis was used to analyze the apparent metabolizable energy content of the adult roosters.

$$Y_{ijk} = \mu + A_i + S_j + (A \times S)_{ij} + e_{ijk}$$

Where:
Y_{ijk}: continuous adjective.
μ: total mean.
A_i: effect of enzymes (i contains 2 levels).
S_j: effect of amaranth in the diet (k contains 4 levels in the regression method).
$(A \times S)_{ij}$: interactive effect of enzymes and amaranth.
e_{ijk}: error term of the experiment.

RESULTS AND DISCUSSION

Feed intake (FI) is one of most significant factors in production cost and in supplying poultry requirements, and nowadays it is the main focus of attention of poultry nutritionists and poultry feed industry manufacturers (McNab and Boorman, 2002). Also, FI is one of the effective parameters for metabolizable energy that can affect energy balance through making reductions in endogenous energy loss (EEL) (McNab and Boorman, 2002). The results of Table 5 reveal that, for the main effects, the level of 60 % RAG had the least feed intake ($P \leq 0.05$). The results of interactions between RAG and enzyme in the parameter of FI show that, with increasing the amaranth level, FI parameter decreased ($P \leq 0.05$).

However, only in level, 60% of RAG with and without enzyme were their significant difference and this level had the least quantity compared to other test groups (P≤0.05). Meanwhile, the reported results in Table 6 show that using PAG for the main effects, at levels of 45% and 60%, the FI reduced significantly (P≤0.05). The results of interactions between PAG and enzyme for FI parameter showed that these interactions for levels of 45% and 60% PAG without enzyme had the least amounts (P≤0.05). Although in other levels, the FI had decreasing trends compared to the control group, there wasn't a significant difference between test groups (P ≥0.05). Decreasing FI for high levels of amaranth in the diet of trial birds could be due to an increase of fiber in the diet, an increase of non-nutrients, alteration of the balance of nutrients, decrease of palatability, and decreased tendency of the birds to eat the diets (Acar *et al*. 1988). Overall, the results of feed intake showed that the use of amaranth (raw and processed) without enzyme ($^{-e}$), could only be used up to 30% for the birds to still eat similar amounts to the control group, and using higher levels of amaranth in the diet resulted in a decrease of FI. If the enzyme was used in diets with RAG, the level of amaranth intake improved up to the level of 45% and was similar to the control group. The impact of the enzyme on the diets with PAG was higher, so intake was similar to the control group up to 60%. Whereas the use of PAG without enzyme ($^{-e}$) was acceptable only up to 30%.

It seems that using an enzyme ($^{+e}$) was more effective in encouraging FI in diets with amaranth because the use of enzymes leads to an effect on the decomposition of non-nutrients, increasing availability of important nutrients like starch, protein, and minerals in cell walls rich in crude fiber and improved diet palatability. Finally**,** it helped the endogenous enzymes of the adult birds and improved FI when high levels of amaranth were offered. The results of this study about the decrease of FI with an increased level of amaranth agreed with reports of Waldroup *et al.* (1985) that had observed the decrease in FI in birds fed high levels of raw amaranth. These researchers, using two species of amaranth (cruentus) and (hypochondriacus) as raw and autoclaved in broiler birds at levels of 20% and 40% in the diet, believed that one of the reasons for the decrease of FI and performance can be due to phenolic compounds and saponins in raw amaranth. This was consistent with the results of the investigations of Connor *et al.* (1980) that reported that high levels of raw amaranth lead to a decrease in FI and performance.

The negligible differences between the results of this study and other reports were due to different levels in intake, bird model, the difference in amaranth variety, and a difference in the processing method.

But overall, the results of this study and other researches showed that adding high levels of amaranth without thermal and enzyme processing certainly results in feed intake reduction, and any methods of processing and also using enzyme can help in improving feed intake on a diet with high levels of amaranth. That agreed with other researches on amaranth.

In general, reliable energy estimation and availability to it is the basis of the correct formulation of the diet in poultry nutrition (McNab and Boorman, 2002). If the value of GE is closer to the value of metabolizable energy (ME), this illustrates the high nutritional value and high- availability of nutrients (Rochell *et al*. 2011). The results of Table 5 showed that gross energy (GE) for RAG's main effects, in levels 15% and 60%, had the least and the highest values respectively (P≤0.05). Results of Table 6 showed that GE values for the main effects had the least value in levels (without PAG) (P≤0.05). Whereas, the results of the main effects of enzyme showed that the level containing enzyme had more GE compared to the groups without enzyme (P≤0.05). The interactions of PAG and enzyme had the least value for GE parameter in control group (P≤0.05). The values of GE in PAG with enzyme ($^{e+}$) had significant an incremental trend, so that levels 45% and 60% had the highest values of GE (P≤0.05). It seems that thermal and enzyme processing led to an increase of GE in the assay diets.

In energy partition discussion, the AMEn has been accepted for poultry. If a feed is not edible by itself or is processed, apparent metabolizable energy is measured by replacing some part of the basal diet with the test ingredient. Based on different percentages of test ingredient replacement, it is possible to write regression equations in any level of use of the test diet for predicting energy values. As apparent metabolizable energy for basal diet is always constant, any energy change in test diets can be attributed to the test ingredient (McNab and Boorman, 2002). Determination of AMEn using the regression method based on replacement method was suggested by Matterson *et al.* (1965) for the first time, and later many studies and researches were developed by other researchers for determining the metabolizable energy of ingredients in poultry by different protocols (e.g poultry model, time, feeding number, different ingredients, etc.) (Pesti *et al*. 2005). Sibbald and Walynets (1989) reported that AMEn estimates a more exact estimation of test ingredients of energy.

Results of this study showed that increasing RAG level with and without enzyme could increase the AMEn values but there were no significant differences in received content of AMEn by birds for the main effects and also for interactions (P≥0.05).

Table 5 Effect of different levels of raw amaranth grain (RAG) with ($^{+e}$) and without ($^{-e}$) enzyme replacement on the energy balance of Leghorn adult rooster in the first experiment

Item	Feed intake (g)	Gross energy intake (kcal)	Energy excretion (kcal)	AME_n (kcal/kg)	Metabolisability (AME_n/GE) (%)	Digestibility dry matter (%)
Raw amaranth grain (RAG) (%)						
% 0	289.71^{a}	3784.25^{ab}	3017.44	3323.58	87.83^{ab}	84.81^{ab}
% 15	287.96^{a}	3451.14^{b}	3076.40	3098.62	89.69^{a}	88.65^{a}
% 30	289.47^{a}	3536.05^{b}	3190.57	3163.84	89.47^{a}	88.04^{a}
% 45	284.74^{a}	3794.24^{ab}	3132.62	3287.35	86.72^{b}	83.68^{b}
% 60	268.32^{b}	3995.63^{a}	3334.01	3434.76	85.81^{b}	8253^{b}
SEM	2.85	148.20	157.71	138.16	0.89	1.33
P-value	0.0001	0.045	0.672	0.463	0.014	0.0096
Enzyme ($^{\pm e}$)						
($^{-e}$)	281.90	3637.96	3086.78	3189.44	88.03	85.20
($^{+e}$)	286.18	3786.56	3213.64	3333.83	87.78	85.88
SEM	1.80	93.73	99.74	87.38	0.56	0.84
P-value	0.104	0.271	0.376	0.252	0.84	0.571
Raw amaranth grain × enzyme (RAG$^{\pm e}$)						
(% 0 RAG^{-e})	291.54^{a}	3641.97	2959.35	3232.81	88.71^{abc}	86.09^{ab}
(% 15 RAG^{-e})	284.22^{ab}	3365.80	2961.93	3021.02	89.63^{ab}	87.57^{a}
(% 30 RAG^{-e})	285.81^{a}	3478.25	3133.20	3124.44	89.80^{a}	88.19^{a}
(% 45 RAG^{-e})	283.08^{ab}	3764.83	3074.38	3224.52	85.88^{bc}	88.22^{b}
(% 60 RAG^{-e})	264.86^{c}	3938.97	3305.03	3344.40	84.87^{c}	80.94^{b}
(% 0 RAG^{+e})	287.86^{a}	3926.54	3075.53	3414.35	86.09^{bc}	83.54^{ab}
(% 15 RAG^{+e})	291.70^{a}	3365.80	3190.86	3176.22	89.75^{ab}	88.72^{a}
(% 30 RAG^{+e})	293.12^{a}	3593.86	3247.95	3203.24	89.14^{ab}	87.89^{a}
(% 45 RAG^{+e})	286.40^{a}	3823.65	3190.86	3350.18	87.57^{abc}	85.14^{ab}
(% 60 RAG^{+e})	271.79^{bc}	4052.28	3362.98	3525.13	86.76^{abc}	84.13^{ab}
SEM	4.03	209.58	223.04	195.38	1.26	1.88
P-value	0.016	0.986	0.997	0.999	0.0462	0.032

AMEn: apparent metabolizable energy corrected for nitrogen and GE: gross energy.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

AMEn values for the main effects of PAG were the least value for level 0% (without PAG). Levels 30%, 45% and 60% had the highest values (P≤0.05). Also, for the main effects of the enzyme, the level containing enzyme had more values of AMEn compared to the group without enzyme (P≤0.05).

Results of interactions between PAG and enzyme ($^{+e}$) showed that the AMEn of the control group had the least value (P≤0.05). In levels containing PAG with and without enzyme ($^{-e}$), AMEn values in PAG level 60% without enzyme ($^{-e}$), and PAG levels 60%, 45%, and 60% with an enzyme ($^{+e}$), had significant differences and the most values (P≤0.05).

Use of RAG with an enzyme ($^{+e}$) led to AMEn values similar to the control group. Also, thermal processing and use of enzyme resulted in an increase of AMEn and a significant difference with the control group and improved energy and gross energy availability (GE) for the birds. The value of AME in the diet, in addition to nutrients and non-nutrients, depends on factors such as age and kind of processing of test ingredient (Larbier and Leclercq, 1994).

Also, Bedford and Partridge (2001) believed that increasing FI increased AMEn, because when FI is high, endogenous effects decline. Janmohammadi *et al.* (2005) reported that factors such as calcium and fat in the ingredient diet can lead to interactions and can affect metabolizable energy values. Choct and Annison (1992) and Annison *et al.* (1996) and suggested that using multi-enzymes in most cereal grains in poultry nutrition can help in cell wall decomposition, nutrient digestion, and AMEn improvement. They agreed with the present research in enzyme effect on PAG that increased AMEn. However, Kalmendal and Tauson (2012) using multi-enzyme in poultry diet didn't find any significant effect on AMEn, and this result was consistent with the enzyme results on RAG in assay diets. However, multi-enzyme intake is dependent on factors like diet ingredients, age of poultry and food processing (Bedford and Apajalahtind, 2001).

Table 6 Effect of different levels of processing amaranth grain (PAG) with ($^{+e}$) and without enzyme ($^{-e}$) replacement on energy balance of Leghorn adult rooster in second experiment

Item	Feed intake (g)	Gross energy intake (kcal)	Energy excretion (kcal)	AME_n (kcal/kg)	Metabolisability (AMEn/GE) (%)	Digestibility dry matter (%)
Processing amaranth grain (PAG) (%)						
% 0	287.26^{a}	3800.66^{c}	3420.21	3336.67^{b}	87.70^{a}	86.29^{a}
% 15	282.29^{ab}	4288.49^{b}	3449.19	3659.79^{ab}	85.27^{ab}	81.56^{b}
% 30	281.68^{ab}	4490.16^{ab}	3478.16	3807.69^{a}	84.60^{ab}	80.19^{bc}
% 45	273.83^{b}	4718.37^{ab}	3448.47	3920.00^{a}	83.10^{b}	76.95^{bc}
% 60	271.37^{b}	4921.05^{a}	3478.31	4094.37^{a}	83.46^{ab}	76.57^{c}
SEM	3.77	152.70	146.60	152.85	1.38	1.57
P-value	0.034	0.001	0.998	0.018	0.016	0.001
Enzyme ($^{\pm e}$)						
($^{-e}$)	279.34	4211.23^{b}	3362.52	3613.07^{b}	85.84	82.30^{a}
($^{+e}$)	279.23	4676.26^{a}	3547.22	3914.34^{a}	83.81	78.23^{b}
SEM	2.38	96.57	92.71	96.67	0.87	0.99
P-value	0.973	0.001	0.169	0.035	0.112	0.008
Processing amaranth grain × enzyme (PAG$^{\pm e}$)						
(% 0 PAG^{-e})	288.27^{a}	3637.20^{e}	3304.46	3231.91^{c}	88.67^{a}	87.52^{a}
(% 15 PAG^{-e})	282.89^{abc}	4039.40^{cde}	3364.13	3447.20^{bc}	85.19^{ab}	82.16^{ab}
(% 30 PAG^{-e})	289^{a}	4268.61^{cde}	3362.41	3630.87^{abc}	84.78^{ab}	80.73^{ab}
(% 45 PAG^{-e})	266.87^{c}	4439.30^{bcd}	3361.55	3770.68^{abc}	84.89^{ab}	80.45^{ab}
(% 60 PAG^{-e})	269.69^{bc}	4671.67^{abcd}	3420.07	3984.69^{ab}	85.68^{ab}	80.64^{ab}
(% 0 PAG^{+e})	286.26^{ab}	3964.12^{de}	3535.97	3441.43^{bc}	86.73^{ab}	85.07^{ab}
(% 15 PAG^{+e})	281.69^{abc}	4537.58^{abcd}	3534.25	3872.39^{abc}	85.35^{ab}	80.96^{ab}
(% 30 PAG^{+e})	274.37^{abc}	4711.72^{abc}	3593.92	3984.51^{ab}	84.43^{ab}	79.64^{bc}
(% 45 PAG^{+e})	280.78^{abc}	4997.45^{ab}	3535.40	4069.32^{ab}	81.31^{b}	73.45^{cd}
(% 60 PAG^{+e})	273.05^{abc}	5170.43^{a}	3536.55	4204.06^{a}	81.25^{b}	72.50^{d}
SEM	5.34	215.95	207.32	216.16	1.95	2.23
P-value	0.014	0.019	0.998	0.009	0.017	0.036

AMEn: apparent metabolizable energy corrected for nitrogen and GE: gross energy.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In the present experiment, equations of prediction were formulated based on different levels of replacement in the diet from 0% to 60% by the regression method, and in Figures 2, 3, 4, and 5 equations and confidence limits (R^2), raw amaranth without enzyme (RAG^{-e}), raw amaranth with an enzyme (RAG^{+e}), processed amaranth without enzyme (PAG^{-e}) and processed amaranth with an enzyme (PAG^{+e}) respectively.

If (x) is replaced by amaranth substitution percent, we can obtain (y) that represents AMEn. If in all introduced equations, x is replaced by the value 100, AMEn values are calculated that results show that AMEn values for RAG^{-e}, RAG^{+e}, PAG^{-e}, PAG^{+e} based on regression equations, were respectively 3250.24, 3433.18, 3242.10, 3438.09 and confidence limits (R^2) for these parameters were 87, 95, 71, 85 percent respectively.

Connor *et al.* (1980) using amaranth grain species edulis reported that the AMEn of raw amaranth grain is based on dry matter 88.4%, the values of AMEn for raw grain and autoclaved grain were reported 3145 and 3775 kcal/kg, respectively.

In summary, the AMEn values for RAG by Connor *et al.* (1980), Acar *et al.* (1988), and Ravindran *et al.* (1996) were 3145, 3210, and 2832 kcal/kg respectively. Also, the AME_n values for PAG by Connor *et al.* (1980), Tillman and Waldroup (1986), Laovoravit *et al.* (1986), Acar *et al.* (1988), Tillman and Waldroup (1988) and Ravindran *et al.* (1996) were 3745, 2859, 3475, 2860, 3650, 3040, 3522 and 3133 kcal/kg respectively.

In detailing the reports, Laovoravit *et al.* (1986) determined the AME and TME of amaranth species cruentus using adult broiler cockerels by method of Vohra *et al.* (1982), the value of AME were in range of 2762 to 3038 kcal/kg.

Tillman and Waldroup (1988), in a study named assessment of extruded grain amaranth as a feed ingredient for broilers, estimated the values of AMEn in grain amaranth species cruentus using two methods.

These two methods were: 1) total collection method of excreta (TCM) and use of acid-insoluble ash (AIA) as a marker using regression analysis and also the difference method.

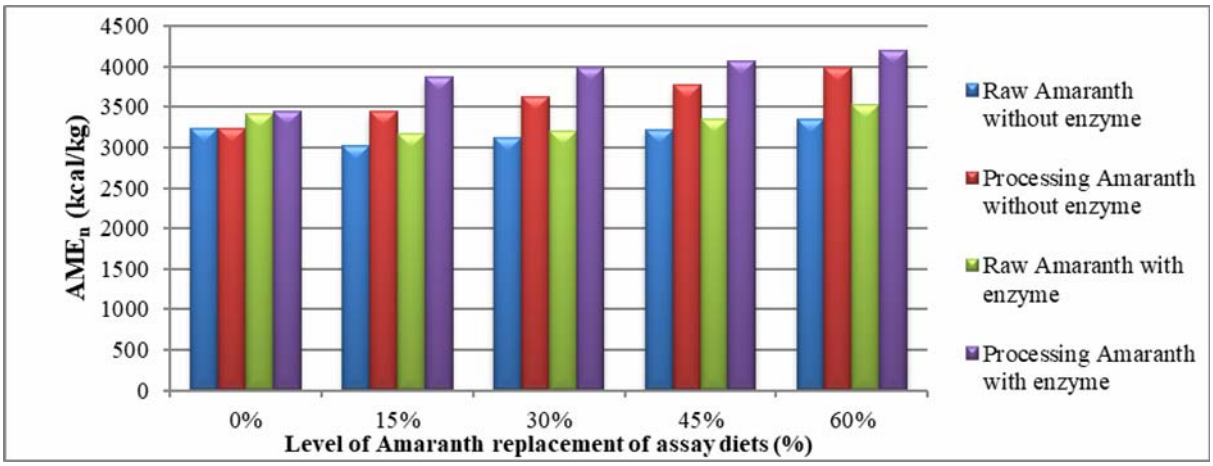

Figure 1 AMEn content in assay diet

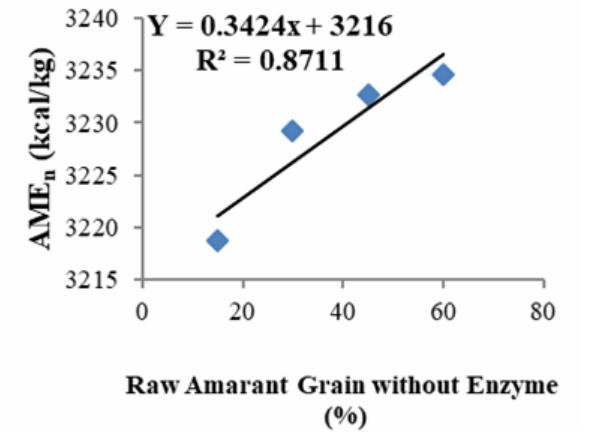

Figure 2 AMEn content of experimental diets assayed of raw aamaranth grain without enzyme (RAG^{-e})
Note: when x= 100%, the AMEn value is 3250.24 kcal/kg

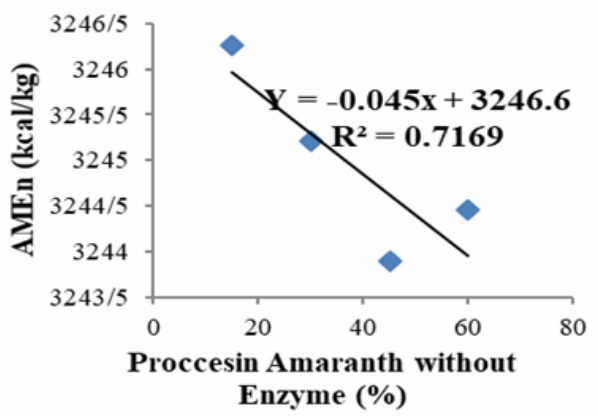

Figure 4 AMEn content of experimental diets assayed of processing aamaranth grain without enzyme (PAG^{-e})
Note: when x= 100%, the AMEn value is 3242.10 kcal/kg

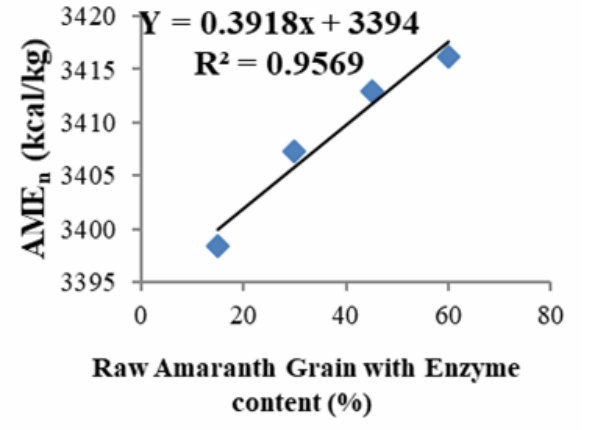

Figure 3 AMEn content of experimental diets assayed of raw aamaranth grain with enzyme (RAG^{+e})
Note: when x= 100%, the AMEn value is 3433.18 kcal/kg

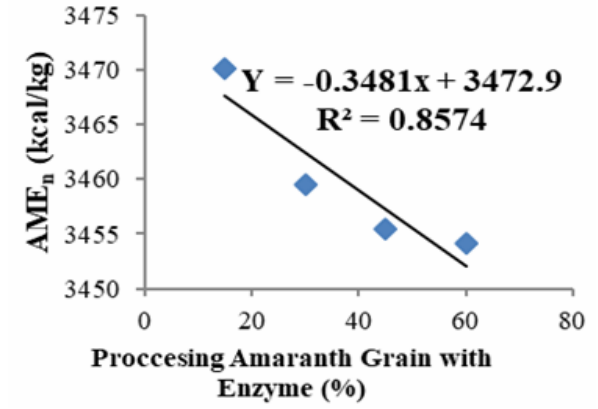

Figure 5 AMEn content of experimental diets assayed of Processing Aamaranth Grain with an enzyme (PAG^{+e})
Note: when x= 100%, the AMEn value is 3438.09 kcal/kg

Overall, results showed that the TCM method develops more exact and more appropriate results compared to the AIA method, because the values of R^2 were more in TCM and that is illustrative of higher accuracy of prediction. Using regression prediction equations in TCM methods, the AMEn value was 3522 kcal/kg based on dry matter. When the difference method was used for calculation of the ME of extruded grain, with the AMEn value based on dry matter, these AMEn values were reported as 3415 kcal/kg.

Acar *et al.* (1988) by nutritional evaluation of grain amaranth for growing chickens, reported that AME values based on dry matter for raw amaranth as flour, fat-free flour, milled containing perisperm, milled with bran and popped forms, were 3210, 3090, 3680, 3060 and 2980 kcal/kg respectively. AME values of the above-mentioned forms for autoclaved amaranth were 3040, 2940, 3100, and 3170 respectively.

Tillman and Waldroup (1986) reported the metabolizable energy value based on dry mater as 3650 kcal/kg, using bioassay. The ME value for Amaranth species edilus that had been under thermal processing, was 3475 kcal/kg based on dry matter (Connor *et al*. 1980).

Ravindran *et al.* (1996) evaluated the AMEn of raw and autoclaved amaranth by broiler chickens using the classical method of Mollah *et al.* (1983). The period of this experiment was seven days for adaptation to assay diets, 3 days for using assay diets and 1-day fasting. At the end of the experiment day, urine and excretion of the four final days were collected. Finally, AME values were calculated based on dry matter and its value for raw and autoclaved amaranth was 2832.21 and 3133.36 kcal/kg, respectively.

Results of this study and other researches show that the AMEn content of grain amaranth was in the range of 2800-3600.

Possible reasons for some of the numerical differences were due to differences in variety, processing methods and the breed of birds and differences in biological experimental methods.

Final assessment and analysis and overview of the range of energy values showed that the results of AMEn in this research were in agreement with other researches.

Table 7 shows the comparison of two kinds of Amaranth (raw and autoclaved) at a similar level, with and without enzyme. Data analysis and to compare the mean treatments were performed using the T-test. The results of the report in Table 7 showed that without enzyme treatments, AMEn values processing amaranth was 60% higher than raw amaranth ($P<0.05$). Treatments with the enzyme at all levels were significantly different and AMEn values processing amaranth was higher than raw amaranth ($P<0.05$). By comparing average PAG^{-e} with aerage RAG^{-e}, it was revealed that the content AMEn of amaranth was improved and increased on average 16.6% due to processing. Comparing average PAG^{+e} with average RAG^{+e} resulted in an increase in content AMEn by 17.78% due to processing. Also, according to Figure 1, AMEn results show that the thermal processing of amaranth and also the use of enzyme resulted in a significant improvement of AMEn value.

According to the diversity of anti-nutritional compounds in energizing and protein feed sources like Amaranth, it seems that adding a mixture of enzymes (multi-enzyme) to the diet could be more helpful and economical compared to individual enzymes.

Metabolisability represents the efficiency of use of nutrients energy that is obtained by mathematical dividing of AMEn to GE. The higher this value, the higher the quality of nutrients in terms of energy availability (Pesti *et al.* 2005). Level zero and 15% RAG without enzyme ($^{-e}$), and also all levels of RAG with enzyme ($^{+e}$), had the highest values of metabolic ability ($P\leq0.05$). Results of interactions between PAG and enzyme showed that the levels containing PAG 45% and 60% with enzyme had the least metabolisability compared to the control group ($P\leq0.05$). It seems that birds fed with RAG containing enzyme ($^{+e}$) had the highest metabolisability. Due to a decrease in nutrients balance, the use of higher levels of amaranth resulted in a decrease of metabolisability but use of enzyme resulted in an increase of metabolisability. In this study, the enzyme roles were in agreement with other studies related to enzyme effect on reduction of anti-nutrient effects, such as reduction of non-starch poly saccharides like xylans that exist in annual plants like amaranth, the role of a drop in viscosity, an increase of endogenous activity of lipase and chymotrypsin enzymes, improvement of digestibility of dry matter and protein and improvement of apparent metabolizability of energy was shown accurately (Bedford and Apajalahti, 2001).

Measurement of the digestibility dry matter parameter depends on the weight of FI and excreta weight based on percent of dry matter (DM). In Table 5, results of digestibility dry matter for interactions between RAG and enzyme showed that RAG levels 15 % and 30 %, without enzyme ($^{-e}$) had higher and significantly different values of digestibility dry matter compared to test groups RAG levels 45% and 60% without enzyme ($^{-e}$) ($P\leq0.05$). But, when the enzyme was used ($^{+e}$), this difference was eliminated and when using amaranth in higher levels, digestibility of dry matter improved.

These results were consistent with the results of the research of Bedford and Apajalahti (2001) that reported that the use of multi-enzyme in diets containing some cereal grain with anti-nutrients in poultry feed results in an improvement of the digestibility dry matter.

Table 7 Comparison of AMEn content different levels of raw amaranth (RAG) and processing amaranth (PAG) with and without enzymes in adult leghorn roosters

Amaranth × enzyme	AMEn of raw amaranth grain (RAG) (kcal/kg)	AMEn of processing amaranth grain (PAG) (kcal/kg)	P-value	Percentage increase of energy PAG than RAG
15 × 0	3021.02	3447.20	0.18	14.11 (%)
30 × 0	3124.44	3630.87	0.22	16.21 (%)
45 × 0	3224.52	3770.68	0.14	16.94 (%)
60 × 0	3344.40[b]	3984.69[a]	0.01	19.15 (%)
				Average= 16.6 (%)
15 × 0.055	3176.22[b]	3872.39[a]	0.03	21.91 (%)
30 × 0.055	3203.24[b]	3984.51[a]	0.04	24.39 (%)
45 × 250	3350.18[b]	3770.68[a]	0.04	12.56 (%)
60 × 0.055	3525.13	4204.06	0.15	12.01 (%)
				Average= 17.78 (%)

AMEn: apparent metabolizable energy corrected for nitrogen.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

In Table 6, the results of the interactions of PAG and enzyme for digestibility dry matter showed that the control group had the highest value and the group containing PAG 45% and 60% with an enzyme ($^{+e}$) had the least values (P≤0.05). Reduction of digestibility of dry matter resulted from the use of high levels of processed amaranth due to alterations in the nutrients balance, reduction of enzyme effect and decrease in anti-nutrient effects.

CONCLUSION

The use of amaranth in this study showed that it can be useful in poultry nutrition and had a good prospect. By studying chemical composition and determining AMEn content and other parameters, it can be concluded that the Amaranth used in this study can be complemented with common cereal like corn and wheat, in levels less than 60%. Regardless of the cost of producing amaranth grain and enzyme, supplementation of the poultry diet with raw and processed amaranth with enzyme can supply the poultry requirement to AMEn, with high confidence. AMEn values of this study were similar and closer to maximum values of other reports for different species and varieties. The highest, "confidence limits" (R^2) were for RAG^{+e} that can estimate AMEn value with higher accuracy. Research and development of amaranth grain and enzyme can be effective in economic decision making and the production strategies of poultry nutritionists and product managers.

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