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The Energy Value for Broiler Chickens of Heat-Treated and Untreated Amaranth Grain, with and without Enzyme Addition

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Abstract: Amaranth is a pseudocereal which can thrive in conditions of drought and limited inputs. Samples of amaranth grain were subjected to proximate analysis with standard laboratory methods. We conducted two experiments to determine apparent (corrected to zero nitrogen balance) metabolisable energy (AME_n) content of untreated (UAG) and heat-treated (HTAG) amaranth grain for Ross-308 male broiler chicks (35–42 d and BW 2141 ± 10.41 g). In each experiment, 10 assay diets (ADs) were fed to 400 birds in individual metabolism cages in a 2 × 5 factorial design. ADs were obtained by substituting amaranth for the main ingredients in the reference diet (RD). Two levels of enzyme addition (0 and 0.55 g/kg) and five amaranth replacement rates (0, 150, 300, 450 and 600 g/kg) were used, and metabolism trials were conducted using the total excreta collection method. Two regression equations were estimated for UAG, with and without enzyme addition, that determined the AME_n content of UAG as 3264 and 3255 kcal/kg, respectively. For HTAG, the AMEn contents with and without enzyme addition were 3973 and 3828 kcal/kg, respectively. Thus, enzyme addition improved the energy value of UAG and HTAG by 0.28 and 3.8%, respectively. The AME_n value of HTAG was 708 and 573 kcal/kg higher than UAG in diets with and without enzyme addition, respectively. Thus, we conclude that there was more benefit from heat treatment than enzyme addition, but there was a synergistic effect of heat treatment and enzyme inclusion on the metabolisable energy concentration of amaranth in the diets of broilers.

Keywords: amaranth grain; climate change; drought; metabolisable energy; enzyme supplements

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1. Introduction

Global climate change is expected to increase the exposure of grain crops to elevated temperatures, by 1–4 °C by the year 2100, and to increase the prevalence of droughts, at the same time as demand for chicken meat is increasing rapidly to meet the growth in world population and increased affluence in many developing countries [1]. Amaranth is a widespread, dicotyledonous, pseudocereal, some genera of which have been cultivated for thousands of years in Central and South America [2], whereas others are considered weeds. In Aztec culture, amaranth was used as a staple crop and had religious significance, but the Spanish forbade its cultivation in an attempt to suppress their culture [3]. Amaranth responds positively to temperature increases of 4 °C, up to 32 °C [4], as well as having high yields and drought resistance by virtue of its C4 photosynthetic pathway [5]. Amaranth

has a water requirement half that of corn and is recommended in areas where sorghum and millet are grown [6]. Moreover, fertilizer prices have more than doubled in the last two years [7], considerably more than grain prices, which means that crops with low nitrogen requirements such as amaranth have to be carefully considered. There are no reliable statistics on worldwide amaranth cultivation; however, it is mainly grown in China and Russia [8], with 300,000 and 100,000 ha reported to be under cultivation around the turn of the century [9]. It is also grown in Central and South America, India and parts of Africa. With international trade worth approximately 1 billion USD, the world's major exporter is China (14% of the global market), followed by the Netherlands (8%) and Austria (7%) [10]. Different amaranth varieties are grown for two main products: grain (*Amaranthus cruentus*, *A. hybridus* and *A. hypochondriacus*) and a green vegetable (*A. tricolor* and *A. lividus*). Both have high-potential and multipurpose usage [11], including as an ingredient in bakery products and as a thickening agent. There are also non-food uses, such as dusting powder in cosmetics [9]. The starch from amaranth grain has a low gelatinisation temperature and is stable over a wide range of temperatures, making it suitable for use as a thickening agent [9]. Amaranth protein is of better amino acid composition for humans than most major cereals and even cows' milk [9]. Amaranth contains more oil than the other major cereals, with a saturated fat-to-unsaturated fat ratio of about 0.3. Amaranth also has some antinutritive factors as well as polyphenols with antioxidant properties, which are claimed to reduce blood cholesterol [8]. The antioxidant properties are amplified by heat treatment, probably as a result of increased flavonoids, whereas phenolic content is variably affected, depending on variety and type of heat treatment [12]. Viscosity is typically increased by heat treatment; however, protein digestibility declines [12]. Heat treatment may be in the form of autoclaving, popping or roasting.

Amaranth has a fast growth rate, and typical yields of amaranth grains per hectare are between 2 and 6 tons [9], providing there is sufficient heat for germination and for the first three months of growth. It can be used in rotation with winter wheat. Amaranths are considered a relatively neglected species that is important for future food security [13–15].

The main use of amaranth grain in livestock diets has been in China, where it is used extensively, as is silage from green material [9]. As well as a high nutritive value, it has significant antibacterial and antiviral effects [16]. It is recommended as a supplement for humans in developing countries, particularly to reduce the incidence of anaemia by virtue of its high iron content [16]. Fermented amaranth is a good substrate for probiotics [17], but prefermenting prior to feeding to livestock has not yet been evaluated for its effects on the nutritive value of amaranth grain. The nutritive value of amaranth grain (*A. cruentus*, *A. hypochondriacus* and *A. caudatus*) as both raw flour and the extruded product has been evaluated in monogastric animals [18]. Its value for poultry was studied quite extensively in the 1980s [19–25]. More recently, different species of amaranth grain (*A. cruentus* and *A. hypochondriacus*) as untreated and heat-treated (autoclaved and extruded) forms have been used in growth, feeding and metabolism trials in broilers, laying hens, quails and turkeys [18,26–32]. The results of these studies demonstrate that inclusion of amaranth grain in broiler diets at low levels (≤200 g/kg) results in comparable performance to diets based on a corn–soybean meal mixture. Higher levels of inclusion of amaranth, up to 400 g/kg, have had no adverse effects on production or carcase traits of laying hens or broilers provided that the amaranth was autoclaved or extruded [20,22,23,25,28,32].

The AME_n values for raw and autoclaved grain of *A. edulis* have been determined as 2780 and 3072 kcal/kg on an as-fed basis [33]. The AME_n value of extruded grain of *A. cruentus*, determined by regression in the study of Tilman and Waldroup [21], was 3267 kcal/kg feed. Ravindran et al. [25] reported 2832 and 3133 kcal/kg of AME for untreated and autoclaved *A. hypochondriacus* grain, respectively. Different TME_n values of amaranth grain have been detected for chickens and turkeys [26].

Enzyme preparations are used by the poultry feed production industry to improve the available energy content of diets containing cereal grains and plant protein meals [34–37]. Different enzymes, for example α-amylase or amyloglucosidase, have variable effects on

the physicochemical properties of amaranth starch, but generally improve its resistance to crystallisation and broaden the range of gelatinisation temperatures [38]. The α-amylases from a proprietary thermostable bacteria product were more effective than those from *Bacillus subtilis*, which were in turn more effective than those from the fungus *Aspergillus oryzae*. However, the effects of enzymes on amaranth grain's available energy content have not been evaluated for poultry. Therefore, the objective of the present study was to determine the chemical composition and AME_n content of raw and autoclaved forms of *Amaranthus hybridus chorostachys* with and without multienzyme addition in adult male broilers.

2. Materials and Methods

The chickens were raised under standard husbandry conditions in the poultry research unit belonging to the Animal Science Department, Faculty of Agriculture, University of Tabriz.

2.1. Birds and Management

A total of 800 one-day-old male Ross-308 broiler chickens (400 birds for each of two separate experiments) were obtained from a commercial hatchery (breeding reference: Arta. Ltd., Ardebil, Iran) and raised on a litter floor at the brooding and rearing unit. Vaccination programs were carried out in accordance with the recommendations of the General Veterinary Administration of the region. The lighting program was 23 h light and 1 h dark daily. The temperature at placement was 34 °C, which was reduced gradually to 20 °C using thermostatically controlled heaters and fans to ensure comfort for the birds. Corn–soybean meal diet with balanced nutrient requirements, in accordance with the Ross-308 recommendations, were fed ad libitum during starter and grower phases. The birds had free access to water.

In each experiment, a week before starting a metabolism trial at day 35, 400 birds with mean body weight of 2141 ± 10.41 g were selected and transferred to metabolic cages. To accurately measure feed consumption and faecal output, individual metabolic cages (40 cm × 65 cm × 75 cm) were used with stainless steel feeders, watering cups and faecal collection pans. In order to determine AME_n content in the metabolism trials, two separate bioassays were conducted, the first with untreated amaranth grain (UAG) and the second with heat-treated amaranth grain (HTAG) in a 2 × 5 factorial design with ten dietary treatments and ten replicates, each of four birds (400 birds per experiment). Two levels of enzyme addition, 0 ($^{-E}$) and 0.55 ($^{+E}$) g/kg, and five levels of amaranth grain (0, 150, 300, 450 and 600 g/kg) were used as the two factors. By inclusion of different levels of $UAG^{\pm E}$ and $HTAG^{\pm E}$ with corn grain in the reference diet (RD) (Table 1), 20 assay diets (ADs) were obtained. In the ADs, the main ingredients (corn, soybean meal, barley and vegetable oil) in RD were replaced by amaranth grain ($UAG^{\pm E}$ and $HTAG^{\pm E}$), so that the ratios between the non-amaranth ingredients were unchanged.

Table 1. Ingredients and nutrient composition of the reference diet (RD) fed to birds from 35 to 42 d of age.

Ingredients	Content, g/kg
Corn Grain	570.3
Soybean Meal (SBM)	321.1
Barley	53.7
Vegetable Oil	16.0
Di-Calcium-Phosphate	14.7
$CaCO_3$	12.6
Na-Bicarbonate	3.2
NaCl	1.8
DL-Methionine	1.6
Vitamin and Mineral Premix [1]	5.0

Table 1. *Cont.*

Ingredients	Content, g/kg
Calculated nutrient composition	
AME_n, kcal/kg	2950
Crude Protein, g/kg	190
Calcium, g/kg	7.8
Available Phosphorus, g/kg	3.8
Sodium, g/kg	1.4
DCAB, mEq/kg	250
Calculated total (digestible) amino acid content, g/kg	
Arginine	12.6 (12.0)
Lysine	10.1 (9.2)
Threonine	7.4 (6.5)
Methionine	2.8 (2.6)
Methionine + Cystine	5.9 (5.2)
Tryptophan	2.3 (2.1)
Glycine + Serine	17.9 (15.9)
Valine	9.1 (8.1)
Isoleucine	8.2 (7.5)
Leucine	16.4 (15.2)
Histidine	5.1 (4.8)
Phenylalanine	9.5 (8.8)
Phenylalanine + Tyrosine	16.2 (15.1)

[1] Vitamin and mineral premix includes, per kilogram of diet: 7200 IU vitamin A (transretinyl acetate); 1.75 mg thiamin (thiamin mononitrate); 6.6 mg riboflavin; 8 mg nicotinic acid; 2.5 mg pyridoxine (pyridoxine HCl); 18 ug vit B12 (ciano cobalamin; 2500 IU vit D3 (cholecalciferol); 15 mg pantothenic acid (D-calcium pantothenate); 0.4 mg D-biotin; 16 IU vit E (Dl-α-tocopherol acetate); 2.5 mg vit K (menadione); 1 mg folic acid; 400 mg choline chloride; 80 mg manganese oxide; 36 mg iron; 9 mg sulphate copper; 0.85 mg iodine; 5 mg selenium (selenium premix); 90 mg Zn (zinc oxide).

Chemical analysis and GE measurement of diets and total excreta followed methodology described previously [25,39]. RD and ADs were fed to birds for two days for adaptation. Then, after 24 h fasting, RD and ADs were fed again for 72 h followed by 24 h of fasting. Total excreta output during these four days were collected in plastic zip-keep bags and stored in a freezer at −20 °C.

2.2. Test Ingredients

The included test ingredients in ADs were amaranth grain as untreated (UAG) and heat-treated (HTAG) forms. After herbarium evaluation in the National Botanical Garden (Tehran, Iran), the species *Amaranthus hybridus chlorostachys* was cultivated in the Khalat-Poushan Educational and Research Center, Faculty of Agriculture, University of Tabriz, in the year 2018, planted in June and harvested in September.

Grains were separated, sifted and milled by a threshing machine (Arian.Eng 211, Arian Agricultural Machines Co, Tehran, Iran), cleaning machine (A.R.S 500, Alborz Industrial Machines Co, Karaj, Iran) and stone mill (ML218, Ghorbani Electrical Machines Co, Rasht, Iran), respectively, in the Darvash Giah Khazar Medicinal Herbs Complex. For heat treatment, grains underwent autoclave processing, at 120 °C for 5 min. The milled grains were transferred to the Advanced Animal Nutrition Lab where chemical analysis was performed.

2.3. Chemical Analyses

Chemical analysis of feed and excreta samples was according to the methods detailed by Association Official Analytical Chemist [40]. Frozen samples of collected excreta were placed at 70 °C for 72 h in a drying Oven Memmert (Model:100–800, made in Germany), and after cooling, moisture exchange for 24 h (under laboratory conditions) and separation of feathers, they were weighed and milled.

Nitrogen (N) content of feed and excreta samples were measured by a Foss 2300 Kjeletc Analyzer. Gross energy (GE) was determined by a Parr adiabatic bomb calorimeter (Parr 6100 Instruments Co., Moline, IL, USA), using benzoic acid as a standard calibration. The amount of crude protein (CP) was calculated as N × 6.25. In addition, ether extract (EE) was analysed by solvent extractor (Ser 148, Analytical Instrument Velp Scientifica), crude fibre (CF) by Fibertec (Ser 1010, Foss Analytic), Ash by Electric furnace Shin Seang (Model: SEF-201). In addition, neutral-detergent fibre (NDF) and acid detergent fibre (ADF) were measured by Van Soest et al.'s method [41].

2.4. Statistical Analysis

Linear regression was used to determine AME_n values of the amaranth grain treatments. In the series of ADs, the test ingredient was incorporated into RD in amounts from 0 (RD) to 600 g/kg, respectively, in 15% increments, so that five ADs were produced for UAG^{-E}, UAG^{+E}, $HTAG^{-E}$ and $HTAG^{+E}$, respectively.

The AME_n was calculated as follows: AME_n = [(Feed intake × GE_{diet}) − (Excreta output × $GE_{excreta}$) − (NR × K)]/Feed intake [42].

Where NR (nitrogen retention) = (Feed intake × Feed nitrogen content) − (Excreta output × Excreta nitrogen content). K = 8.22 kcal/g NR [43].

In order to determine the AME_n of the test ingredient, linear relationships between the values of AME_n of the ADs and the intake of the test ingredient were evaluated [44]. In this way, the AME_n intake associated with amaranth intake was regressed against the intake of amaranth. The linear equation obtained (Y = ax + b) was used to estimate AME_n for a diet containing 100% amaranth grain.

Percentages of dry matter digestibility (DMD) and metabolisability were calculated as follows: DMD = [(Feed DM intake − Excreta DM output)/Feed DM intake] × 100, and Metabolisability = (AME_n/GE) × 100 [22].

Random errors were evaluated for normal and independent distribution and homogeneity of variance using the Shapiro–Wilk test and Bartlett's test, respectively. Experimental data were analysed using a completely randomized design as the factorial arrangement within R software [45,46]. Data were expressed as mean values ± SEM, and the differences were compared with Duncan's multiple range test [47]. Differences were assumed to be significant if $p < 0.05$.

3. Results

The chemical composition and GE content of amaranth grain (*A. hybridus chlorostachys*) are presented in Table 2. The differences between most UAG and HTAG chemical constituents were small; however, NDF contents and NFE were both increased by heat treatment.

Table 2. Chemical compositions of amaranth grains (*A. hybridus chlorostachys*) on an as-fed basis.

Specification, g/kg	UAG [1]	HTAG [2]
DM	909	904
CP	169	168
EE	57	52
CF	106	101
NDF	339.7	348.2
ADF	61.9	68.4
Ash	61	56
NFE [3]	607	623
NFC [4]	373	376
GE, kcal/kg	4277	4258

[1] Untreated amaranth grain. [2] Heat-treated amaranth grain. [3] Nitrogen-free extract (NFE) = 1000 − (CP + EE + CF + Ash). [4] Nonfibrous carbohydrates (NFC) = 1000 − (CP + EE + NDF + Ash).

3.1. Untreated Amaranth Grain

The main effects of UAG level, enzyme addition and their interaction on AME_n, metabolisability and DMD in ADs are shown in Table 3. Amaranth inclusion reduced AME_n at the lowest inclusion rate, but thereafter increased it. Similarly, metabolisability initially decreased at the lowest level, but above 300 increased with each increment of amaranth inclusion. DM digestibility was reduced for all levels of amaranth inclusion, compared with the zero inclusion rate.

Table 3. AME_n, metabolisability and dry matter digestibility (DMD) values of broiler chickens fed different levels of untreated amaranth grain (UAG) with ($^{+E}$) and without ($^{-E}$) enzyme from 35 to 42 d of age in the second experiment.

Factor	AME_n, kcal/kg	Metabolisability, %	Dry Matter Digestibility (DMD), %
Untreated amaranth grain (UAG), g/kg			
0	2869 ^{b}	75.59 ^{a}	66.65 ^{a}
150	2606 ^{d}	70.17 ^{c}	61.11 ^{c}
300	2732 ^{c}	70.12 ^{c}	60.10 ^{c}
450	2889 ^{b}	72.45 ^{b}	63.02 ^{b}
600	3189 ^{a}	74.99 ^{a}	59.83 ^{c}
SEM	20.83	0.39	0.63
p-value	0.0001	0.0001	0.0001
Enzyme ($^{\pm E}$), g/kg			
0 ($^{-E}$)	2848	70.70	59.75
0.55 ($^{+E}$)	2866	74.62	64.53
SEM	13.17	0.25	0.40
p-value	0.35	0.0001	0.0001
UAG × Enzyme			
0 × 0	2789 ^{cd}	74.15 ^{bc}	65.86 ^{ab}
150 × 0	2693 ^{e}	70.39 ^{d}	60.20 ^{d}
300 × 0	2723 ^{de}	67.23 ^{e}	56.48 ^{e}
450 × 0	2812 ^{c}	69.13 ^{d}	60.69 ^{d}
600 × 0	3224 ^{a}	72.63 ^{c}	55.54 ^{e}
0 × 0.55	2949 ^{b}	77.04 ^{a}	67.44 ^{a}
150 × 0.055	2518 ^{f}	69.95 ^{d}	62.01 ^{cd}
300 × 0.55	2742 ^{cde}	73.01 ^{c}	63.73 ^{bc}
450 × 0.55	2966 ^{b}	75.78 ^{ab}	65.35 ^{ab}
600 × 0.55	3154 ^{a}	77.35 ^{a}	64.12 ^{bc}
SEM	29.46	0.56	0.88
p-value	0.001	0.001	0.019

^{a,b,c,d,e,f} means within each column with different superscripts differ significantly ($p < 0.05$).

Enzyme addition had no effect on AME_n but increased both metabolisability and DM digestibility. There were significant interactions between the amaranth inclusion rate and enzyme inclusion on all three variables. AME_n increased more with enzyme inclusion at no or low amaranth inclusion rates. However, metabolisability tended to be increased more by enzyme addition at the higher amaranth inclusion rates, and DMD was reduced less at high amaranth inclusion rates when the enzyme was added.

3.2. Heat-Treated Amaranth Grain

The main effects of the HTAG level, enzyme addition and their interaction on AME_n, metabolisability and DMD are shown in Table 4. AME_n increased from the 0 and 150 inclusion rates, which did not differ, incrementally at higher inclusion rates. The 600 g/kg inclusion rate had an AME_n of 3788 compared with 3098 for 0/150 inclusion rates.

The metabolisability of the diet tended to increase with inclusion rate above 300 g/kg, and the DM digestibility declined from 0 to 300 g/kg and then increased at higher rates.

Table 4. AME_n, metabolisability and dry matter digestibility (DMD) values of broiler chickens fed different levels of heat-treated amaranth grain (HTAG) with ($^{+E}$) and without ($^{-E}$) enzyme from 35 to 42 d of age in the first experiment.

Factor	AME_n, kcal/kg	Metabolisability, %	Dry Matter Digestibility (DMD), %
Heat-treated amaranth grain (HTAG), g/kg			
0	3060 ^{d}	76.74 ^{c}	67.11 ^{a}
150	3136 ^{d}	75.83 ^{cd}	65.04 ^{b}
300	3251 ^{c}	74.97 ^{d}	59.57 ^{d}
450	3556 ^{b}	78.66 ^{b}	63.28 ^{bc}
600	3788 ^{a}	79.94 ^{a}	62.69 ^{c}
SEM	27.86	0.39	0.66
p-value	0.0001	0.0001	0.0001
Enzyme ($^{\pm E}$), g/kg			
0 ($^{-E}$)	3329	76.11	62.36
0.55 ($^{+E}$)	3397	78.35	64.72
SEM	17.62	0.24	0.42
p-value	0.002	0.0001	0.011
HTAG × Enzyme			
0 × 0	3021 ^{f}	75.62 ^{e}	64.62 ^{b}
150 × 0	3107 ^{ef}	75.14 ^{e}	65.59 ^{b}
300 × 0	3236 ^{d}	72.49 ^{f}	63.54 ^{b}
450 × 0	3514 ^{c}	78.22 ^{bcd}	63.54^{b}
600 × 0	3720 ^{b}	79.08 ^{bc}	62.56 ^{b}
0 × 0.55	3098 ^{ef}	77.86 ^{bcd}	69.60 ^{a}
150 × 0.55	3166 ^{de}	76.53 ^{de}	64.49 ^{b}
300 × 0.55	3266 ^{d}	77.44 ^{cd}	63.65 ^{b}
450 × 0.55	3597 ^{c}	79.10 ^{b}	63.03 ^{b}
600 × 0.55	3855 ^{a}	80.80 ^{a}	62.83 ^{b}
SEM	39.40	0.55	0.94
p-value	0.032	0.004	0.007

^{a,b,c,d,e,f} means within each column with different superscripts differ significantly ($p < 0.05$).

Enzyme addition increased AME_n, metabolisability and DM digestibility. The interaction between the two factors, heat treatment and enzyme addition, which were significant for all the variables, showed that there was a synergistic effect of amaranth inclusion at the highest level and enzyme inclusion, with AME_n increasing from 3720 without enzyme to 3855 with enzyme. Similarly, metabolisability was greater with enzyme addition at the highest amaranth inclusion rate (80.8 vs. 79.1). However, DM digestibility was similar for all treatments except at the zero amaranth inclusion rate, when enzyme addition increased the DMD from 64.6 to 69.6%.

The regression analysis of AME_n values of amaranth grain as untreated and heat-treated forms in diets with/without enzyme addition on intake values of amaranth grain resulted in four prediction equations. The equations are presented in Table 5. All equations showed relatively high determination coefficient values (r^2), from 98 to 99%. The solution of the four equations for x = 1 (100% substitution of amaranth grain in RD) resulted in four AME_n (kcal/kg) values, which were as follows: 3255, 3265, 3829 and 3973 for UAG^{-E}, UAG^{+E}, $HTAG^{-E}$ and $HTAG^{+E}$, respectively. The average value of AME_n, 3580 kcal/kg, represented 84% of GE content. Overall, the processing of untreated amaranth grain with enzyme and heat treatment improved AME_n values by 10 and 573 kcal/kg, respectively.

Table 5. Regression equations relating $UAG^{\pm E}$- and $HTAG^{\pm E}$-associated AME_n content to intake of ADs.

Treatment [1]	Regression Equation [2]	r^2
UAG^{-E}	Y = 3359.4 (118.76) x − 104.05 (32.88)	0.98
UAG^{+E}	Y = 3370.3 (46.04) x − 105.75 (12.76)	0.99
$HTAG^{-E}$	Y = 3939.8 (75.92) x − 111.01 (21.02)	0.99
$HTAG^{+E}$	Y = 4102.7 (85.12) x − 129.28 (23.58)	0.99

[1] UAG^{-E}: untreated amaranth grain without (−) enzyme, UAG^{+E}: untreated amaranth grain with (+) enzyme, $HTAG^{-E}$: heat-treated amaranth grain without (−) enzyme, $HTAG^{+E}$: heat-treated amaranth grain with (+) enzyme. [2] Numerical values in parentheses are SE, intercepts are in kcal, line slopes are in kcal/kg of DM. X: amaranth grain intake (%).Y: AME_n from amaranth grain intake (kcal/kg).

4. Discussion

4.1. Nutritional Value of Amaranth

The values of CP, EE and CF in amaranth grain were higher than those in cereals. The high value for GE can be attributed to the high content of organic matter (850 g/kg) in amaranth grain. NDF was also high in the ADs, which probably decreased DMD in ADs containing a high level of amaranth grain. However, the increased NDF and ADF in amaranth grain was not enough to cause a decrease in the availability of energy in the ADs.

Nutrient concentrations in amaranth seed have been determined previously [9,19,48,49]. The concentrations of nutrients (CP, EE, CF, NFE and NFC) in our study were mostly within the range of reported values. Compared with our value of 3255 kcal/kg, UAG has previously been found to have AME_n values of 3145 kcal/kg [33], 2859 kcal/kg [20] and 2832 kcal/kg [25]. AME_n values for *A. hypochondriacus, A. cruentus* and *A. hybridus* have been reported to be between 2700 and 3600 kcal/kg [9]. Connor et al. [33] reported AME_n values of untreated and autoclaved amaranth species (*A. edulis*) as 3145 and 3475 kcal/kg, respectively. Tillman and Waldroup [22] determined the AME_n values of extruded *A. cruentus* in broiler chickens as 3522 and 3415 kcal/kg DM, respectively. Ravindran et al. [25] estimated the AME_n value of *A. hypochondriacus, either* untreated or autoclaved, in broiler chickens as 2832 and 3133 kcal/kg dry matter, respectively. Acar and Vohra [20] reported that the AME value based on the DM for untreated amaranth in the forms of flour, fat-free flour, milled containing perisperm, milled with bran and popped were as follows: 3210, 3090, 3680, 3060 and 2980 kcal/kg, respectively. The AME of these forms for autoclaved amaranth were 3040, 2940, 3100 and 3170 kcal/kg, respectively.

In comparison with the report of Cai et al. [9], *A. hybridus chlorostachys* in our study had less CP (168 g/kg) than the corresponding value for *A. hypochondriacus* and twice the CF content (101 g/kg) compared with *A. hybridus*, *A. cruentus* and *A. caudatus* (60, 40 and 50 g/kg, respectively). The high CF content would probably decrease the available energy from *A. hybridus chlorostachys* for poultry. However, Dhellot et al. [49] evaluated the chemical composition of *A. hybridus* and reported that it was a rich source of protein (170 g/kg), which is consistent with the present study. The CP value for amaranth seeds in the present study was in the range of CP values for amaranth seeds in different species (140–170 g/kg) [50]. Ravindran et al. [25] reported CP, EE, CF and ash concentrations of 168, 58, 60 and 26 g/kg, respectively, for *A. hypochondriacus*. These are close to the corresponding values in this study, except for CF and ash. Amaranth grain is two times richer in CP and EE than corn, which is the basic energy provider in RD.

The digestibility of dry matter (DM) in monogastric animals, particularly poultry that have short and less sacculated intestinal tracts, is greatly affected by the fibre fraction in the feed. As shown in Table 1, the NDF value in amaranth grain is high, containing cellulose and hemicellulose that are indigestible by broilers.

4.2. Heat Treatment

The increase in DMD with heat treatment suggests that this could be used to improve the efficiency of nutrient digestibility. The improvement in AME_n content could have re-

sulted from an increasing contribution of energy-producing compounds in diets containing HTAG, in comparison with the control diet. There are reports in the fields of food and feed science that suggest heat treatment can reduce negative effects on the energetic efficiency of the grains [51]. Previous estimates of AME_n in HTAG were 2859 [32], 3040 [20], 3133 [25], 3522 [23], 3650 [22] and 3475 kcal/kg [21,33]. Differences are probably due to differences in amaranth variety, physicochemical properties and amaranth-processing methods. Raw amaranth contains acids and nitrate that can be reduced with heat treatment [25]; hence, autoclaved amaranth grain can be used in broiler diets for improved energy availability without any negative effects on health. UAG also contains antinutritive compounds such as tannins, phytic acid, oxalate, saponins, nitrates and trypsin inhibitors [52]. For this reason, heat treatment of amaranth grain may be beneficial, potentially reducing phytic acid by up to 20% [52], with consequent increase in the availability of energy and other nutrients.

4.3. Enzyme Addition

Exogenous enzymes are used for improving energy availability in poultry and swine diets. Different enzyme preparations are available for poultry feed, and their effectiveness depends on bird age, enzyme level, nature of basal diet fed and also the physical and chemical characteristics of enzymes. In the present study, the enzyme inclusion increased DMD, but there was no significant improvement in AME_n values.

In our study, the highest values for metabolisability were found in the diets containing enzymes. Generally, the diets with enzyme addition showed a higher value of DMD. Unlike HTAG, AME_n in ADs containing UAG was not affected by enzyme addition, and by increasing UAG levels, DMD was decreased significantly ($p < 0.01$). The main effect of the enzyme addition was to increase metabolisability and DMD by nearly 5 and 8%, respectively.

The effect of the enzyme on the cell wall of grains is to increase digestion of other nutrients and the possible improvement of AME_n [53,54]. Enzymes can reduce antinutritive compounds, non-starch polysaccharides (NSP) [55–57] and viscosity [54,58] in both cereal and pseudocereals such as amaranth. Enzymes potentially improve the activity of lipase and chymotrypsin enzymes [59] in poultry, and consequently they can enhance DMD [60–62] and improve AME_n [63–65]. However, the efficiency of the multienzyme additions to the diet depends on several factors such as bird age, method of processing of test ingredient and the composition and formulation of the diet [66]. In our study, HTAG showed the greatest benefit from enzyme addition at high amaranth inclusion rates, suggesting synergistic effects of enzyme and heat treatment of amaranth, that may be due to the greater opportunities for enzymatic activity when nutrients are released by heat treatment. UAG showed no such clear benefit of enzyme provision at high amaranth inclusion rates.

4.4. Experimental Method

The experimental method in this study was based on regression analysis, the advantage of which is that it can estimate energy values across several inclusion rates [22,67]. The observed AME_n values were in line with those reported by Cai et al. [9] of between 2700 and 3600 kcal/kg. Differences in variety, processing method, animal model and method of biological experiments are likely to cause discrepancies in AME_n values across different studies. The use of the regression method in this study does not adequately take into account any non-linear trends in the measured parameters' responses to increasing the amaranth inclusion rate. Such trends were evident for several parameters, but they require elaboration with further experimentation with greater bird numbers. Of particular interest is the trend for greatest improvements at high inclusion rates, with little effect at low rates, such as was observed for the metabolisability of the diet and DM digestibility.

5. Conclusions

Amaranth, having twice the CP content and an AME_n value equal to corn grain in the processed form, offers considerable potential to poultry producers in regions suffering

from water shortage or restrictions in other inputs. Using enzyme and heat treatment simultaneously for processing untreated amaranth grain improved energy value by up to 718 kcal/kg, equivalent to 22% of the AME_n content of UAG (3255 kcal/kg).

Amaranth grains are mostly considered as an energy source replacement for corn. The AME_n value of amaranth grain (*A. hybridus chlorostachys*) was 230 and 470 kcal more than corn and wheat grains, respectively. This additional energy in amaranth grain for poultry, coupled with the reduced water requirement during the growing period, could help significantly in dealing with water shortages, providing a feed of superior nutritional value, drought and dehydration resistance and resilience to environmental stress conditions [5], provided the feeding value is realizable.

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References

1. Whitton, C.; Bogueva, D.; Marinova, D.; Phillips, C.J.C. Are We Approaching Peak Meat Consumption? Analysis of Meat Consumption from 2000 to 2019 in 35 Countries and Its Relationship to Gross Domestic Product. *Animals* **2021**, *11*, 3466. [CrossRef]
2. Ruth, O.; Unathi, K.; Nomali, N.; Chinsamy, M. Underutilization Versus Nutritional-Nutraceutical Potential of the *Amaranthus* Food Plant: A Mini-Review. *Appl. Sci.* **2021**, *11*, 6879. [CrossRef]
3. Rastogi, A.; Shukla, S. Amaranth: A New Millennium Crop of Nutraceutical Values. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 109–125. [CrossRef] [PubMed]
4. Hwang, S.-G.; Chao, H.-C.; Lin, H.-L. Differential Responses of Pak Choi and Edible Amaranth to an Elevated Temperature. *HortScience* **2018**, *53*, 195–199. [CrossRef]
5. Jamalluddin, N.; Massawe, F.J.; Mayes, S.; Ho, W.K.; Singh, A.; Symonds, R.C. Physiological Screening for Drought Tolerance Traits in Vegetable Amaranth (*Amaranthus tricolor*) Germplasm. *Agriculture* **2021**, *11*, 994. [CrossRef]
6. Kauffman, C.; Weber, L. Grain amaranth. In *Advances in New Crops*; Simon, J.E., Ed.; Timber Press: Portland, OR, USA, 1990; pp. 127–139.
7. World Bank 2022. Fertilizer Price Index. Available online: https://ycharts.com/indicators/fertilizers_index_world_bank (accessed on 16 September 2022).
8. Taylor, J.R.N.; Emmambux, M.N. Products containing other speciality grains: Sorghum, the millets and pseudocereals. In *Technology of Functional Cereal Products*; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing: Sawston, UK, 2008; Chapter 13; pp. 281–339.
9. Cai, Y.; Corke, H.; Wu, H. Amaranth. In *Encyclopedia of Grain Science*; Wrigley, C.W., Corke, H., Walker, C.E., Eds.; Elsevier; Academic Press: Amsterdam, The Netherlands, 2004.
10. Amaranth Seed Global Market Overview Today—Tridge. Available online: www.tridge.com/intelligences/amaranth-seed (accessed on 23 September 2022).
11. Hoidal, N.; Gallardo, M.D.; Jacobsen, S.-E.; Alandia, G. Amaranth as a Dual-Use Crop for Leafy Greens and Seeds: Stable Responses to Leaf Harvest Across Genotypes and Environments. *Front. Plant Sci.* **2019**, *10*, 817. [CrossRef]

12. Muyonga, J.H.; Andabati, B.; Ssepuuya, G. Effect of heat processing on selected grain amaranth physicochemical properties. *Food Sci. Nutr.* **2013**, *2*, 9–16. [CrossRef]
13. FAO; UNICEF. *The State of Food Security and Nutrition in the World 2017: Building Resilience for Peace and Food Security*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2017.
14. FAO; IFAD; UNICEF; WFP; WHO. *The State of Food Security and Nutrition in the World 2020. Transforming Food Systems for Affordable Healthy Diets*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2020.
15. Li, X.; Siddique, K.H. *Future Smart Food: Rediscovering Hidden Treasures of Neglected and Underutilized Species for Zero Hunger in Asia*; Food and Agriculture Organization of the United Nations (FAO): Bangkok, Thailand, 2018.
16. Baraniak, J.; Kania-Dobrowolska, M. The Dual Nature of Amaranth—Functional Food and Potential Medicine. *Foods* **2022**, *11*, 618. [CrossRef]
17. Ugural, A.; Akyol, A. Can pseudocereals modulate microbiota by functioning as probiotics or prebiotics? *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 1725–1739. [CrossRef]
18. Peiretti, P.G.; Meineri, G.; Gai, F.; Longato, E.; Amarowicz, R. Antioxidative activities and phenolic compounds of pumpkin (*Cucurbita pepo*) seeds and amaranth (*Amaranthus caudatus*) grain extracts. *Nat. Prod. Res.* **2017**, *31*, 2178–2182. [CrossRef]
19. Waldroup, P.W.; Hellwig, H.M.; Longer, D.E.; Endres, C.S. The Utilization of Grain Amaranth by Broiler Chickens. *Poult. Sci.* **1985**, *64*, 759–762. [CrossRef]
20. Acar, N.; Vohra, P.; Becker, R.; Hanners, G.; Saunders, R. Nutritional Evaluation of Grain Amaranth for Growing Chickens. *Poult. Sci.* **1988**, *67*, 1166–1173. [CrossRef]
21. Tillman, P.; Waldroup, P. Processing Grain Amaranth for Use in Broiler Diets. *Poult. Sci.* **1986**, *65*, 1960–1964. [CrossRef]
22. Tillman, P.B.; Waldroup, P.W. Assessment of Extruded Grain Amaranth as a Feed Ingredient for Broilers: 1. Apparent Metabolizable Energy Values. *Poult. Sci.* **1988**, *67*, 641–646. [CrossRef]
23. Tillman, P.; Waldroup, P. Performance and Yields of Broilers Fed Extruded Grain Amaranth and Grown to Market Weight. *Poult. Sci.* **1988**, *67*, 743–749. [CrossRef] [PubMed]
24. Vohra, P.; Bersch, S.; Acar, N.; Becker, R. Use of grain amaranth in diets of Japanese quail and chickens. *Nutr. Rep. Int.* **1989**, *39*, 1227–1235.
25. Ravindran, V.; Hood, R.; Gill, R.; Kneale, C.; Bryden, W. Nutritional evaluation of grain amaranth (*Amaranthus hypochondriacus*) in broiler diets. *Anim. Feed Sci. Technol.* **1996**, *63*, 323–331. [CrossRef]
26. Jacob, J.P.; Noll, S.L.; Brannon, J.A. Comparison of Metabolic Energy Content of Organic Cereal Grains for Chickens and Turkeys. *J. Appl. Poult. Res.* **2008**, *17*, 540–544. [CrossRef]
27. Jakubowska, M.; Gardzielewska, J.; Tarasewicz, Z.; Szczerbinska, D.; Karamucki, T.; Rybak, K.; Polawska, E.; Garczewska, J. The effect of amaranth seed added to the standard diet upon selected meat quality traits in the quail. *Anim. Sci. Pap. Rep.* **2013**, *31*, 355–362.
28. Popiela, E.; Kroliczewska, B.; Zawadzki, W.; Opalinski, S.; Skiba, T. Effect of extruded amaranth grains on per-formance, egg traits, fatty acids composition, and selected blood characteristics of laying hens. *Livest. Sci.* **2013**, *155*, 308–315. [CrossRef]
29. Szczerbinska, D.; Pyka, B.; Szabelska, E.; Ligocki, M.; Majewska DRomaniszyn, K.; Sulik, M. The effect of diet with amaranth (*Amaranthus cruentus*) seeds on Japanese quail (*Coturnix coturnix japonica*) performance, somatic development, hatching results and selected blood biochemical parameters. *Vet. Med. Zootech.* **2015**, *70*, 67–72.
30. Ríos, H.R.; Parra, J.C.; Neira, R.A.; Cano, J.G.; Domínguez, S.C.; Gil Romo, F.P. *Amaranthus cruentus* L. as a food alternative in laying hens to reduce cholesterol in eggs. *Chil. J. Agric. Anim. Sci.* **2020**, *36*, 78–85. [CrossRef]
31. Alizadeh-Ghamsari, A.H.; Hosseini, S.A.; Soleymani, M.R.; Nahavandi, R. Performance, Intestinal Histomorphology, and Blood Variables of Broilers Fed Amaranth Grain in Pellet Diet. *Trop. Anim. Sci. J.* **2021**, *44*, 71–78. [CrossRef]
32. Laovoravit, N.; Kratzer, F.H.; Becker, R. The Nutritional Value of Amaranth for Feeding Chickens. *Poult. Sci.* **1986**, *65*, 1365–1370. [CrossRef]
33. Connor, J.; Gartner, R.; Runge, B.; Amos, R. *Amaranthus edulis*: An ancient food source re-examined. *Aust. J. Exp. Agric.* **1980**, *20*, 156–161. [CrossRef]
34. Cho, J.H.; Zhao, P.; Kim, I.H. Effects of emulsifier and multi-enzyme in different energy density diet on growth performance, blood profiles, and relative organ weight in broiler chickens. *J. Agri. Sci.* **2012**, *4*, 161.
35. Kalvandi, O.; Janmohammadi, H.; Geshlagh, O.M. Effect of enzyme supplementation on metabolizable energy and apparent nitrogen retention in broiler diets based on corn-soybean meal. *Anim. Sci. Res.* **2015**, *25*, 65–78.
36. Gitoee, A.; Janmohammadi, H.; Taghizadeh, A.; Rafat, S. Effects of a multi-enzyme on performance and carcass characteristics of broiler chickens fed corn-soybean meal basal diets with different metabolizable energy levels. *J. Appl. Anim. Res.* **2014**, *43*, 295–302. [CrossRef]
37. Moftakharzadeh, S.A.; Janmohammadi, H.; Taghizadeh, A.; Kianfar, R.; Olyayee, M.G. Effect of enzyme addition on energy utilization and performance of broiler chickens fed wheat-based diet with different metabolizable energy levels. *Acta Sci. Anim. Sci.* **2018**, *41*, 44585. [CrossRef]
38. Gorinstein, S.; Lii, C.-Y. The Effects of Enzyme Hydrolysis on the Properties of Potato, Cassava and Amaranth Starches. *Starch* **1992**, *44*, 461–466. [CrossRef]
39. Dale, N.; Fuller, H. The use of true metabolizable energy (TME) in formulating poultry rations. *Proc. Georgia Nutr. Conf.* **1981**, 50–57.

40. AOAC (Association of Official Analytical Chemists) International. *Official Methods of Analysis*; AOAC: Washington, DC, USA, 1990.
41. Van Soest, P.; Robertson, J.; Lewis, B. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccha-rides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
42. Sibbald, I.R.; Wolynetz, M.S. Research Note: Pellet Binder and Steam Pelleting as Nitrogen-Corrected True Metabolizable Energy Contributors: An Example of the Statistics Used to Evaluate a Component of a Mixture. *Poult. Sci.* **1989**, *68*, 1299–1302. [CrossRef]
43. Hill, F.W.; Anderson, D.L. Comparison of Metabolizable Energy and Productive Energy Determinations with Growing Chicks. *J. Nutr.* **1958**, *64*, 587–603. [CrossRef]
44. Adeola, O.; Ileleji, K. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. *Poult. Sci.* **2009**, *88*, 579–585. [CrossRef] [PubMed]
45. Demétrio, C.G.; Menten, J.F.; Leandro, R.A.; Brien, C. Experimental power considerations—Justifying replication for animal care and use committees. *Poult. Sci.* **2013**, *92*, 2490–2497. [CrossRef] [PubMed]
46. Team, R.C. *R: A Language and Environment for Statistical Computing. Manual*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
47. Duncan, D.B. Multiple Range and Multiple *F* Tests. *Biometrics* **1955**, *11*, 1–42. [CrossRef]
48. Yanez, E.; Zacarias, I.; Granger, D.; Vasquez, M.; Estevez, A.M. Chemical and nutritional characterization of ama-ranthus (*Amaranthus cruentus*). *Arch. Latinoam. Nutr.* **1994**, *44*, 57–62.
49. Dhellot, J.; Matouba, E.; Maloumbi, M.; Nzikou, J.; Ngoma, D.S.; Linder, M.; Desobry, S.; Parmentier, M. Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (var 1 and 2) of Congo Brazzaville. *Afr. J. Biotechnol.* **2006**, *5*, 1095–1101.
50. Downtown, W. *Amaranthus edulis*: A high lysine grain amaranth. *World Crop.* **1973**, *25*, 20–25.
51. Blair, R. *Nutrition and Feeding of Organic Poultry*; CABI: Wallingford, UK, 2018.
52. Gamel, T.H.; Linssen, J.P.; Mesallam, A.S.; Damir, A.A.; Shekib, L.A. Effect of seed treatments on the chemical composition of two amaranth species: Oil, sugars, fibres, minerals and vitamins. *J. Sci. Food Agric.* **2005**, *86*, 82–89. [CrossRef]
53. Singh, A.; Berrocoso, J.D.; Dersjant-Li, Y.; Awati, A.; Jha, R. Effect of a combination of xylanase, amylase and protease on growth performance of broilers fed low and high fiber diets. *Anim. Feed Sci. Technol.* **2017**, *232*, 16–20. [CrossRef]
54. Bedford, M.R. The evolution and application of enzymes in the animal feed industry: The role of data interpretation. *Br. Poult. Sci.* **2018**, *59*, 486–493. [CrossRef] [PubMed]
55. Bedford, M. Mechanism of action and potential environmental benefits from the use of feed enzymes. *Anim. Feed Sci. Technol.* **1995**, *53*, 145–155. [CrossRef]
56. Maurya, N.K.; Arya, P. Amaranthus grain nutritional benefits: A review. J. *Pharmacogn. Phytochem.* **2018**, *7*, 2258–2262.
57. Westbrook, L.A.; Cherian, G. Egg quality, fatty-acid composition and gastrointestinal morphology of layer hens fed whole flaxseed with enzyme supplementation. *Br. Poult. Sci.* **2019**, *60*, 146–153. [CrossRef] [PubMed]
58. Kiarie, E.; Romero, L.; Ravindran, V. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. *Poult. Sci.* **2014**, *93*, 1186–1196. [CrossRef]
59. Engberg, R.M.; Hedemann, M.S.; Steenfeldt, S.; Jensen, B.B. Influence of Whole Wheat and Xylanase on Broiler Performance and Microbial Composition and Activity in the Digestive Tract. *Poult. Sci.* **2004**, *83*, 925–938. [CrossRef]
60. Bedford, M.R.; Classen, H.L. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in im-proved growth rate and food conversion efficiency of broiler chicks. *J. Nutr.* **1992**, *122*, 560–569.
61. Kutlu, H.R.; Saber, S.N.; Kutay, H.; Celik, L.; Yusuf, U.; Nurten, T.; Kutlu, M.; Yucelt, O.; Burgut, A.; Thiery, P. Effect of multi-enzyme produced by a single fungus on growth performance and some carcass parameters of broiler chicks fed on maize-soya based diets. *Kafkas Üniversitesi Vet. Fakültesi Derg.* **2019**, *25*, 221–230.
62. Mohammadigheisar, M.; Kim, H.S.; Kim, I.H. Effect of inclusion of lysolecithin or multi-enzyme in low energy diet of broiler chickens. *J. Appl. Anim. Res.* **2018**, *46*, 1198–1201. [CrossRef]
63. Campasino, A.M. Effects of Varying Nutrient Profiles and Enzyme Supplementation in Broiler Diets on Growth Per-formance and Energy Digestibility. Master's Thesis, Texas A&M University, College Station, TX, USA, 2012.
64. Crouch, A.N.; Grimes, J.L.; Ferket, P.R.; Thomas, L.N.; Sefton, A.E. Enzyme Supplementation to Enhance Wheat Utilization in Starter Diets for Broilers and Turkeys. *J. Appl. Poult. Res.* **1997**, *6*, 147–154. [CrossRef]
65. Abdallh, M.; Ahiwe, E.; Musigwa, S.; Changa, E.; Al-Qahtani, M.; Cadogan, D.; Iji, P. Energy and protein utilization by broiler chickens fed diets containing cottonseed meal and supplemented with a composite enzyme product. *Br. Poult. Sci.* **2020**, *61*, 424–432. [CrossRef] [PubMed]
66. Bedford, M.R.; Partridge, G.G. *Enzymes in Farm Animal Nutrition*; CABI: Wallingford, UK, 2001.
67. Dozier, W.; Kerr, B.; Corzo, A.; Kidd, M.; Weber, T.; Bregendahl, K. Apparent Metabolizable Energy of Glycerin for Broiler Chickens. *Poult. Sci.* **2008**, *87*, 317–322. [CrossRef]