Comparison of feed value of *Amaranthus powellii* Willd. forage to some roughage feeds

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Abstract

The aim of this study was to compare the chemical composition, in vitro digestibility, protein and energy value, roughage value of *Amaranthus powellii* Willd. forage to the most commonly used alfalfa hay and wheat straw in ruminant nutrition.

Amaranthus powellii Willd. forage has the potential to be a third quality roughage according to its relative feed value assessment of 104.55 ± 0.67. In addition, the relative forage quality assessment developed for feeding dairy cattle was 97.73 ± 0.05 for *Amaranthus powellii* Willd. forage, which had a higher the relative forage quality value than alfalfa hay and wheat straw, and it can be used for feeding dairy or fattening cattle. However, it has been generally recommended that Amaranth be given either by grazing at its early flowering time or via silage due to the oxalic acid and nitrate salts. *Amaranthus powellii* Willd. forage should also be determined nitrate, amino acids, and other antinutritional factors before studied in vivo as hay or silage.

Keywords: Amaranth hay, amaranth, alfalfa hay, in vitro bait value, wheat straw

1. Introduction

The world population is predicted to be 9.3 billion people in 2050, and it is perhaps one of the most important problems that the projected population is not only common to humans but to animals as well [1]. As the cereals used in human and animal nutrition are the same, there is a competition for the use of grain. Pseudo-cereals have the same nutritional content as the cereals used in human nutrition, but are not as widely used as corn, rice, and wheat [2]. As alternative feed material, Amaranth can be used in animal nutrition instead of some cereals [3]. Amaranth was known by the Aztecs as a grain equivalent to maize in religious ceremonies, that it can grow in the world any climate or soil condition to produce content-rich in energy and protein, that the seeds and leaves can be eaten by humans and animals, and it is a C_4 dicotyledonous plant suitable for carbon fixation [4-10]. In addition, due to the rich nutritional content, it can be compared with other biomass or biogas plants when its potential as a current topic of research is being determined [11-13].

Amaranth contains a higher level of protein, twice the amount of lysine essential amino acid, more fiber, 5 times more calcium and 20 times more iron compared to other cereals [14]. Amaranth seeds contain 5 to 9% ether extract, approximately 77% unsaturated fatty acids, while linoleic acid (5 to 8%) fatty acid [15, 16]. In addition, Amaranth contains high concentrations of oxalic acid (12 to 30% by dry weight (DW) including the leaves), nitrates (0.21 to 0.74% by DW including the leaves), antitrypsin proteins and temperature variable factors [17, 18]. In terms of health, Amaranth seeds lower cholesterol, increase antioxidant capacity, and are an anticancer, anti-allergenic and antihypertensive agent; they act as food to counter celiac disease and immunodeficiency disorders, and in a methanol solution – with the effect of a peptide called lunasin – they have an antitumor, antihyperlipidemic, antidiabetic and anthelmintic effect. Furthermore, in an aqueous solution, they have an antidiarrheal, antifungal and antimalarial effect [19-22].

Amaranth occurs as *Amaranthus hypochondriacus, paniculatus* and *edulis* grain; *paniculatus*, *spinosus*, *tenuifolius*, *tricolor* leaves; *caudatus* cereal or ornamental plant; *Polygamus*, *gracilis*, *dubius*, *spinosus*, *tenuifolius*, *blitum*, *lividus* and *cruentus* varieties are grown as vegetables, while *Amaranthus retroflexus*, *albus*, *hybridus*, *powellii* and *quitensis* are 60 species known to be weeds [5, 6, 14, 23-25]. In general, the yield of Amaranth seeds per hectare is about 1 to 6 tonnes and the green material is about 70 tonnes [26]. Amaranth is a suitable forage for ruminant animals in terms of high bypass protein or rumen undegraded intake protein through C_4 metabolism [27]. In addition, over 40,000 hectares of Amaranth are cultivated for the roughage requirements of pigs in China, and it is recommended that grazing be assessed 84 days after planting due to the nitrate content [27-30]. *Amaranthus caudatus, cruentus, edulis, dubius, hypochondriacus, hybridus, retroflexus, spinosus, tricolor* seed or seed processing (through moist heat: boiling and slurring and through dry heat: popping and roasting), the leaf feed material or grazing or silage are used to feed fish, rodents, monogastric and ruminant animals [25, 31-45]. Amaranth can be used as an alternative source of high protein to meet the protein requirements of farm animals yielding high. In general, studies on a wild species like *Amaranthus powellii* [46] are related to biological or chemical control [47-49], but no studies have been found on chemical composition, alternative nutrient availability, in vitro digestibility in animal nutrition or roughage. For this reason, the chemical composition, roughage value, use in dairy cattle nutrition, and in vitro digestibility of *Amaranthus powellii* Willd. forage (APF) were determined by comparing it to wheat straw (WS) and alfalfa hay (AH), which are most commonly used for ruminant animals.

The present study aimed to compare WS and AH, which are one of the most commonly used roughage foods used for ruminant animals, with APF that grows wild in all climatic conditions.

2. Materials and Method

The APF, which is the main material of the study, was harvested from Kırşehir Ahi Evran University research and application area after the maturation of the seeds was completed and dried under a ventilated drying oven at 65 ° C for 48 hours. In addition, dry WS and AH were taken from industrial animal husbandry farms. Rumen fluid was taken from three different Belgian Blue-Holstein hybrid steers slaughtered at the Kırşehir Meat and Meat Products Food Marketing Industry and Trade Limited Company, Turkey at the age of 28 months. This collected rumen fluids were mixed, before using, to minimize the error caused by a single animal. These three animals' rumen fluids helped to collect sufficient ruminal fluid to carry out in vitro digestibility study. These animals had been fed on 40 concentrates / 60 roughages and had a live weight of about 650 kg before coming to slaughterhouse. As stated by Filik [50], chemical analyzes (dry matter: DM, organic matter: OM, crude protein: CP and ash contents) of APF, WH and AH were determined according to AOAC [51], Van Soest et al. (the crude fiber: CF, neutral detergent fiber: NDF, acid detergent fiber: ADF and acid detergent lignin: ADL) [52], AOCS (ether extract: EE) [53] procedures and calculated values according to formulas Sniffen et al., (total carbohydrates: TC, hemicellulose: HCel, cellulose: Cel, and nitrogen-free extracts: NFE contents) [54]. The digestibility analysis of the APF, WH and AH were determined according to Menke and Steingass [55] in vitro gas production technique [50].

The means of the total gas values were corrected according to the average values of blind samples [56, 57].

The digestible crude protein (%, DCP) [58] and total digestible nutrient (%, TDN) values [59], digestible energy (Mcal/kg, DE) [60], metabolizable energy (Mcal/kg, ME) [61], net energy-lactation (Mcal/lb, NE_L), net energy-maintenance (Mcal/lb, NE_M), net energy-gain (Mcal/lb, NE_G) [62], net energy-maintenance (MJ/kg, NE_m) and net energy-gain (MJ/kg, NE_g) [63], dry matter intake (Live Weight: LW, DMI %), digestible dry matter (DDM), relative feed value (RFV) [64] and relative forage quality (RFQ) [65] values of the APF, WH and AH were calculated by using chemical analysis results.

The experiment samples were divided into 3 groups (APF, WS, and AH) each group contained 8 replicates, 4 of them for chemical and 4 of them in vitro digestibility trial. For the data statistics, descriptive variables were used for the statistical analysis. Mean, standard error (SE) values and Tukey's multiple range test procedures -excluding of in vitro digestibility data- were calculated using the SPSS [66] (17.0) ® statistical software program package (SPSS Institute Inc., Cary, NC, USA).

3. Results and Discussion

The results and discussion were conducted with other Amaranth species since the evaluation of APF as roughage was not found in any publication. Generally, the amount of ADL and ash percentage increase as the plants became dry hay before harvest. Although WS and AH obtained from industrial livestock farms were used to feed dairy cattle and fatten animals, the quality of both sources of roughage were determined to be low in the present study. *Amaranthus hypochondriacus* seed, plant or leaves have been reported to be a low-quality feed when considered as a source of protein, but may be a good quality feed when processed [67]. On the contrary, Sleugh et al. [68] reported that Amaranth can be a good forage according to their studies examining the chemical composition of *Amaranthus cruentus, hybrid, hybridus* and *hypochondriacus* varieties grown in seven different regions and harvested on six dates. A late harvest of *Amaranthus cruentus* and *hypochondriacus,* instead of an early harvest, decreased the percentage of CP but increased the percentage of ADF and NDF [69]. The CP, ash, EE and NFC percentage values for *Amaranthus hypochondriacus* decreased for the early harvest time, while OM, NDF, ADF and ADL percentage values increased, and the result values for the late harvest time were support to our study [70]. NDF may not be used as a source of energy in ruminant feeds, as some of them may bind to lignocellulose complex or fiber, some of which form ADL. In a study into the values for the average moisture, ash, EE, CF and CP percentage values for the branch and leaves of the *Amaranthus albus, blitoides* and *retroflexus* species, they were determined to be: (7.82, 17.65, 1.05, 32.87 and 8.43); (8.66, 10.66, 1.36, 33.38 and 11.09) and (9.26, 12.08, 0.97, 30.82 and 14.40), respectively [71]. Leukebandara et al. [72] reported that *Amaranthus hybridus*, *caudatus*, *hypochondriacus*, *cruentus* and *dubius* species harvested at different periods have a significant potential for being a good dry season forage crop. The amounts of ash percentage for the Amaranth species harvested on the 110th day of the study were support to those for APF result.

Ehsani et al. [73] attributed the changes in the CP, NDF, ADF and ADL percentage values for *Amaranthus hypochondriacus* to late harvesting. The excess of ADL and ash percentage was explained by the high carbon content from a potential C_4 plant per unit area. These results are comparable with data mentioned for WS and AH. In addition, the NFE and CF values of Smitha Patel et al. [74] support the use of APF. According to Su et al. [75], the results for ash, CP, EE, ADF and NDF of WS have similar values to our study. Şehu et al. [76] reported that the CP, CF, ADF and NDF percentage values for AH were 3.5, 38.1, 51.2 and 84.0, respectively. On the contrary, Bozkurt Kiraz [77] determined the ADF and NDF percentage values for AH to be 33.76 and 40.15, and the RFV value to be 145.34, respectively. Looked at the results from this perspective, in the present study, the high NDF percentage values for the WS and AH used may indicate that they are a good filler feed.

Fazaeli et al. [70] determined the dry matter digestibility (DMD) and organic matter digestibility values (OMD) for *Amaranthus hypochondriacus* first and second harvest time to be 78.92, 66.64 and 75.13, 64.32%, and the DMD and OMD of APF were 59.99 and 41.67, respectively. Similarly, Rahnama and Safaeie [78] determined the mean DMD value of three different varieties of *Amaranthus hypochondriacus* as 68.3%. According to all these results, APF has a low DMD and OMD value compared to *Amaranthus hypochondriacus*. Sarmadi et al. [79] determined the forage quality of *Amaranthus hypochondriacus* grown at different developmental stages (flowering, milk and death stage) and nitrogen levels (120, 180 and 240 kg N/ha). While the ADL percentage, phenolics and methane production continuously increased with time; the CP percentage, digestibility, in vitro ruminal volatile fatty acids and microbial crude protein values decreased. Compared to other Amaranth species, the low digestibility of APF can be explained by the increase in the amount of ADL in its structure due to its late harvest. While the TC values of fresh grass and silage of Amaranthus Plainsman and D136 cultivars were determined to be 674 and 662, and 641 and 647 g kg^{-1} [80], APF, WH and AH were determined to be 79.85, 86.97 and 60.20 g kg^{-1}, respectively, in our present study (Table 1). According to these results, while the total carbohydrate value of

Amaranthus Plainsman and D136 varieties increased in fresh grass and silage, ADL value, structural carbohydrate was higher in dried APF, WS, and AH.

While the NE_L values for Amaranthus Plainsman and D136 cultivars for fresh grass and silage were 4.94 and 5.15, and 4.94 and 5.01 MJ/kg DM according to Seguin et al. [80], in our current study, the APF, WH and AH values were determined to be 3.41, 1.65 and 4.45 MJ/kg DM, respectively.

In the present study, the 24-hour OMD value of wheat straw was determined to be 25.73%. In the study by Şehu et al. [81], which determined the feed value and digestibility of different roughages, the value for 24-hour dry matter loss of wheat straw was similar to our study at 30.40% (Table 2).

Amaranth reduces the nitrogen requirements of cultivated soils, while fertilization can provide more plant growth [82]. The late harvest of *Amaranthus cruentus* and *hypochondriacus*, instead of an early harvest, reduced the CP percentage [27]. Abbasi et al. [83] reported that *Amaranthus hypochondriacus* harvested in 60 days as roughage can be increased by nitrogen fertilization. Karimi Rahjerdi et al. [84] showed that the CP percentage value for green grasses of the Kharkovskiy and Sem varieties of *Amaranthus hypochondriacus* decreased to 13.0 and 14.1, respectively. Dumanoğlu and Geren [85] used different doses of nitrogen (5, 10, 15 and 20 kg ha^{-1}) and phosphorus (5 and 10 kg ha^{-1}) applied to *Amaranthus mantegazzianus* green grass and silage; the N15 and P10 values provided the best plant growth and CP percentage (Table 3).

Amaranthus caudatus exhibited a decreased ash percentage in different developmental stages, while Gross Energy (MJ/kg DM) increased, and early flowering (79d) supported our study [86]. Pond and Lehmann, [34] reported that the lamb alfalfa ration can instead be substituted by 50% *Amaranthus cruentus*, as an energy source. Rahnama and Safaeie

[78] determined the crude oil average of three varieties of *Amaranthus hypochondriacus* as 2.20, while APF, WS and AH were 2.16, 1.22 and 6.55%, respectively. While the chemical composition and nutritional values of all studies support our current study, the APF results show that it has a feed value.

The TDN values for Amaranthus Plainsman and D136 varieties were determined when fresh and as silage to be 532 and 552, and 532 and 538 g kg^{-1} by Seguin et al. [80]. This present study found the values for APF, WH and AH to be 53.47, 52.83 and 67.88%, respectively. TDN (%), DE (MJ/kg), ME (MJ/kg), NE_L (MJ/kg), NE_M (MJ/kg), NE_G (MJ/kg), NE_m (MJ/kg), NE_g (MJ/kg) and CP percentage values were highest in AH, APF and WS, respectively. The high CP percentage also increases the energy value [87] and the value for the digestible ME (MJ/kg DM) supports the calculated energy values (Table 3).

The RFV value for APF, WS and AH decreased linearly respectively and the decrease was statistically significant (P<0.0001). According to the RFV assessment, APF has the potential to be a third-tier roughage (Table 4). On the contrary, Rahnama and Safaeie [78] concluded that three varieties of *Amaranthus hypochondriacus* were prime quality roughage on the RFV scale according to changes occurring at different formation times and that they could be used for feeding 18 to 24 month-old dry cows according to the RFQ scale. In our current study, different results to the Rahnama and Safaeie [78] study may be due to regional and species differences. DM, EE, CF, CP and ash contents of APF used in the study were 94.19, 2.16, 28.14, 4.84 and 13.22%, respectively. This result was supported by Bressani and González [88, 89], who concluded that Amaranth would be a good forage or material for silage, and that heat-treated seeds could be used in poultry feed. In addition, the nutritional values of the branches and leaves of Amaranth, in the study by Bressani [90], support our present study. Abbasi et al. [91] reported that there was no quality silage, but there was potential, depending on the amount of ADL in fresh *Amaranthus hypochondriacus* (44.6 g/kg). Ehsani et al. [73] reported that there may be better quality forage than AH used for feeding ruminant animals. Alfaro et al., [92] reported that substituting 15% with alfalfa leaf flour would not be a problem, but higher levels would reduce daily weight gain, while 60% of dried amaranth plant meal would contribute to increased live weight in animals. According to Şehu et al. [76], the CP, CF, ADF and NDF values of alfalfa hay were determined to be 3.5, 38.1, 51.2 and 84.0%, respectively. On the contrary, Bozkurt Kiraz [77] determined the ADF and NDF values of alfalfa hay to be 33.76 and 40.15, and the RFV value to be 145.34, respectively. In the present study, HP, HS, ADF and NDF values were determined to be 19.59, 41.52, 58.01 and 78.23%, respectively. Although these values show similarity, the higher ADL value decreased the RFV value of WS and AH, and was calculated to be 59.54% and 51.98%, respectively. The present study was used to compare two forage feed samples: good quality, like AH, and low quality, like WS. The poor RFV value of alfalfa can be attributed to the lack of care during harvesting, handling and storage.

According to the RFQ value developed for the feeding of dairy cattle, an APF, AH and WS ranking is available (Table 4). The RFQ value for APF has the highest value at 97.73 ± 0.05; this is a value that allows it to be used for feeding dairy cattle or fattening cattle [69]. Odwongo and Mugerwa [93] reported that up to 40% of Amaranth leaves can be added to the pre-weaning rations for calves. Olorunnisomo [94] used sun-dried corn and *Amaranthus cruentus*, equal mixtures of sun-dried, separate silages and equal mixture silages as a complementary feed for dry sheep during dry periods. Tan et al. [95] reported that the shape time for *Amaranthus retroflexus* and *Chenopodium album* plants and the addition of additives (salt and barley) were not sufficient to make good quality silage. Alegbejo [42] reported that Amaranth leaves may be a good roughage, but the best grazing period is flowering time. Aliyu [96] reported that *Amaranthus hybridus* can be added to the mixed feed as an alternative forage feed during feeding of nursing rabbits.

4. Conclusions

Amaranth is generally considered a human food – or food component – as a source of protein because it contains high levels of crude protein and lysine from essential amino acids. However, Amaranthus powellii is a plant that has not been studied beyond its biological and chemical control, and the current study is perhaps the first resource for its evaluation in terms of animal nutrition. APF compared to WH and AH, has the potential to be a third quality roughage according to its RFV assessment of 104.55 ± 0.67. Our results concluded that APF has the potential to be a forage that can be used in feeding ruminant animals according to nutritional and in vitro digestibility analyses; however, in vivo studies are needed to show the effects on ruminant animals after determining nitrate, amino acids, and other antinutritional factors.

Conflicts of Interest

There are no conflicts of interest to declare.

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Bioethics and Biosecurity Committee Approval

The study complied with an ethics document taken from the Animal Experiments Local Ethics Committee of Kırşehir Ahi Evran University, dated and numbered 08/08/2018-15-3.

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Authors' Contributions

Gökhan Filik and Ayşe Gül Filik designed and conducted this study. Ayşe Gül Filik worked at laboratory for chemical analyses and in vitro trial with completing statistical analysis while Gökhan Filik supervised and coordinated this study. Both authors wrote this manuscript with making critical revision and approved of the final version.

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Table 1. Nutritional content of roughages

Parameters	**N**	***Amaranthus powellii* Willd. Forage ± SE**	**Wheat Straw ± SE**	**Alfalfa Hay ± SE**	**P Value**
DM[1]	4	$941.90^{b} \pm 1.90$	$961.00^{a} \pm 0.70$	$922.90^{c} \pm 1.20$	0.0001
ash [2]	4	$13.22^{a} \pm 0.07$	$7.16^{b} \pm 0.23$	$13.68^{a} \pm 0.05$	0.0001
CP [2]	4	$4.84^{b} \pm 0.46$	$4.47^{b} \pm 0.27$	$19.59^{a} \pm 0.99$	0.0008
EE [2]	4	$2.16^{b} \pm 0.25$	$1.22^{b} \pm 0.60$	$6.55^{a} \pm 0.19$	0.0044
CF [2]	4	$28.14^{b} \pm 0.88$	$31.86^{b} \pm 0.13$	$41.52^{a} \pm 1.12$	0.0030
ADF [2]	4	$37.12^{c} \pm 0.16$	$49.47^{b} \pm 0.35$	$58.01^{a} \pm 0.46$	0.0001
NDF [2]	4	$53.38^{b} \pm 0.45$	$78.77^{a} \pm 2.70$	$78.23^{a} \pm 0.76$	0.0026
ADL [2]	4	$35.06^{b} \pm 0.27$	$35.44^{b} \pm 0.38$	$43.01^{a} \pm 0.50$	0.0012
HCel [2]	4	$16.27^{b} \pm 0.61$	$29.30^{a} \pm 3.05$	$20.22^{ab} \pm 1.25$	0.0372
Cel* [2]	4	$2.06^{b} \pm 0.42$	$14.04^{a} \pm 0.03$	$15.01^{a} \pm 0.96$	0.0011
TC [2]	4	$79.85^{b} \pm 0.30$	$86.97^{a} \pm 1.11$	$60.20^{c} \pm 1.11$	0.0005
NFE [2]	4	$45.81^{a} \pm 0.90$	$51.17^{a} \pm 1.34$	$10.86^{b} \pm 0.01$	0.0001

[1]g/kg of natural material; [2] (%) of dry matter.

DM: dry matter, CP: crude protein, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, EE: ether extract, TC: total carbohydrates, HCel: hemicellulose, Cel: cellulose, NFE: nitrogen free extracts,

[a,b,c] Mean values within the same column with no common superscripts differ significantly ($P < 0.01$).

Table 2. Total amount of gas measured in 24 hours by *in vitro* gas production technique of roughages

Roughages	**N**	**OMD** (%) ± **SE**	**ME** (MJ/kg DM) ± **SE**	**NE$_L$** (MJ/kg DM) ± **SE**	***In vitro* gas production** (IVGP, ml/200 mg DM) ± **SE**
***Amaranthus powellii* Willd. Forage**	4	41.67 ± 0.84	6.27 ± 0.13	3.41 ± 0.10	29.70 ± 0.99
Wheat Straw	4	25.73 ± 1.52	3.77 ± 0.24	1.65 ± 0.17	11.36 ± 1.80
Alfalfa Hay	4	50.66 ± 4.34	7.75 ± 0.70	4.45 ± 0.49	39.26 ± 5.14

OMD: organic matter digestibility, ME: metabolizable energy, NE$_L$: net energy lactation, IVGP: in vitro gas production.

Table 3. Protein and energy values of roughages

Parameters	*Amaranthus powellii* Willd. Forage ± SE	Wheat Straw ± SE	Alfalfa Hay ± SE	P values
DCP (%)	$0.62^b \pm 0.42$	$0.29^b \pm 0.25$	$14.02^a \pm 0.90$	0.0008
TDN (%)	$53.47^b \pm 0.42$	$52.83^b \pm 0.28$	$67.88^a \pm 1.11$	0.0010
DE (MJ/kg)	$9.87^b \pm 0.08$	$9.75^b \pm 0.06$	$11.53^a \pm 0.21$	0.0010
ME (MJ/kg)	$8.09^b \pm 0.07$	$7.99^b \pm 0.04$	$10.27^a \pm 0.17$	0.0010
NE_L (MJ/kg)	$5.00^b \pm 0.04$	$4.93^b \pm 0.03$	$6.48^a \pm 0.11$	0.0011
NE_M (MJ/kg)	$5.28^b \pm 0.05$	$5.21^b \pm 0.04$	$7.04^a \pm 0.13$	0.0010
NE_G (MJ/kg)	$2.27^b \pm 0.05$	$2.19^b \pm 0.04$	$4.02^a \pm 0.14$	0.0010
NE_m (MJ/kg)	$6.49^b \pm 0.08$	$6.37^b \pm 0.05$	$9.77^a \pm 0.31$	0.0016
NE_g (MJ/kg)	$4.91^b \pm 0.06$	$4.81^b \pm 0.04$	$7.80^a \pm 0.29$	0.0018

DCP: digestible crude protein: CP * 0.908-3.77, TDN: total digestible nutrient: 50.41 + 1.04 CP – 0.07CF, DE: digestible energy: 0.04409 * TDN % (50% TDN: 6.40 MJ/kg DM of ME), ME: metabolizable energy: 0.82 * DE, NE_L: net energy-lactation TDN% * 0.01114 – 0.054 (1 Mcal/lb = 2.2046 Mcal/kg), NE_M: net energy-maintenance: TDN% * 0.01318 – 0.132 (1 Mcal/ lb = 2.2046 Mcal/kg), NE_G: net energy-gain: TDN% * 0.01318 – 0.459 (1 Mcal/ lb = 2.2046 Mcal/kg), NE_m: net energy-maintenance: $1.37\ ME – 0.138\ ME^2 + 0.0105\ ME^3 – 1.12$ and NE_g: net energy-gain: $1.42\ ME – 0.174\ ME^2 + 0.0122\ ME^3 – 1.65$.

[a,b,c] Mean values within the same column with no common superscripts differ significantly ($P < 0.01$).

Table 4. Relative feed value and relative feed quality values of roughages

Parameters	***Amaranthus powellii*** **Willd. Forage ± SE**	**Wheat Straw ± SE**	**Alfalfa Hay ± SE**	**P values**
DDM (LW %)	59.99[a] ± 0.12	50.37[b] ± 0.27	43.71[c] ± 0.36	0.0001
DMI (%)	2.25[a] ± 0.02	1.53[b] ± 0.06	1.54[b] ± 0.02	0.0011
RFV	104.55[a] ± 0.67	59.54[b] ± 1.71	51.98[c] ± 0.09	0.0001
RFQ	97.73[a] ± 0.05	65.50[c] ± 1.89	84.68[b] ± 2.23	0.0020

DMI: dry matter intake (Live Weight: LW, %): 120/ [NDF%], DDM: digestible dry matter: 88.9 – [0.779 * ADF%] RFV: relative feed value: [DMD * DMI]/1.29 and RFQ: relative forage quality: [DMI * TDN]/1.23.

[a,b,c] Mean values within the same column with no common superscripts differ significantly (P < 0.01)