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*[*Animal Feed Science and Technology 183 (2013) doi site:
http://dx.doi.org/10.1016/j.anifeedsci.2013.04.012*]*

The definitive version is available at:
La versione definitiva è disponibile alla URL:
[http://www.sciencedirect.com/science/article/pii/S037784011300093X]

Fatty acid profile and nutritive value of quinoa (*Chenopodium quinoa* Willd.) seeds and plants at different growth stages

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a native of the Andean region and belongs to the group of crops known as pseudocereals. It has great potential for improving food for humans and animals. Quinoa was studied to determine its fatty acid (FA) composition, chemical composition, gross energy, *in vitro* dry matter digestibility (IVDMD) and neutral detergent fibre digestibility (IVNDFD) of the seeds and of the plant during growth. Herbage samples were collected six times at progressive morphological stages from the early vegetative to the grain fill stage. The effect of plant ageing was analysed by polynomial contrasts. The chemical composition of quinoa is closely connected to the development of the plant and the quality of crop decreased with increasing morphological stages. Dry matter (DM), organic matter (OM), neutral detergent fibre (NDFom) content increased linearly from the mid vegetative to the grain fill stage, while acid detergent fibre (ADFom) content increased linearly from the first to the last stage. The pattern of FAs in the seed was characterised by three dominant FAs: palmitic acid (PA, $C_{16:0}$), oleic acid (OA, $C_{18:1\ n-9}$) and linoleic acid (LA, $C_{18:2\ n-6}$). Between main FAs of the plant during growth, α-linolenic acid (ALA, $C_{18:3\ n-3}$) was the most abundant FA (from 385 to 473 g/kg of total FA), while LA content, which ranged from 146 to 176 g/kg of total FA, decreased with increasing growth until the shooting stage and then increased, while PA, OA and stearidonic acid ($C_{18:4\ n-3}$) did not show significant differences in their content during growth. Moreover as far as LA content polynomial contrasts showed linear and quadratic effects. IVDMD and IVNDFD decreased linearly with increasing stages and the gross energy content from the early vegetative stage to the mid vegetative stage and then increased from the late vegetative stage to the grain fill stage. The first summer cut of quinoa, whose lipid fraction is rich in ALA and other polyunsaturated FAs, should be performed before shooting, since its nutritional quality deteriorates when cutting is delayed.

Keywords: *Chenopodium quinoa* L.; Growth stage; Lipid; Fibrous fractions; Crude protein, *In vitro* digestibility.

Abbreviations: **ADFom**, acid detergent fibre expressed exclusive of residual ash; **ALA**, α-linolenic acid ; **CP**, crude protein; **EE**, ether extract; **FA**, fatty acid; **FM**, fresh matter; **GE**, gross energy; **IVDMD**, *in vitro* dry matter digestibility; **IVNDFD**, *in vitro* neutral detergent fibre digestibility; **LA**, linoleic acid; **NDFom**, neutral detergent fibre expressed exclusive of residual ash; **OA**, oleic acid; **OM**, organic matter; **PA**, palmitic acid; **PUFA**, polyunsaturated fatty acids; **SA**, stearic acid

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) seed is a human staple food of Andean South America that has received attention because of its high nutritional value, due in particular to the fatty acid (FA) composition (Wood et al., 1993) with a high proportion of unsaturated FA, particularly of oleic acid (OA, $C_{18:1\ n-9}$) and linoleic acid (LA, $C_{18:2\ n-6}$) and its balanced amino-acid spectrum with high methionine (4–10 g/kg DM) and lysine (51–64 g/kg DM) contents (Bhargava et al., 2003).

Quinoa seed has also been used as animal feed. Jacobsen et al. (1997) concluded that quinoa seed has potential as broiler feed, but proportionally should not exceed 150 g/kg of the diet, while they found that dehulling of quinoa slightly improved performance in broilers. Horsted and Hermansen (2007) found that nutrient-restricted, high-producing organic layers are capable of finding and utilising considerable amounts of different feed items (quinoa and other forage crops) from a cultivated foraging area without negative effects on their health and welfare. Improta and Kellens (2001) reported that processing (polishing or washing) of quinoa prior to feeding, or diluting the quinoa with some other available feed are viable options that can be considered for improving performance of broilers when quinoa is a major component of the diet.

Quinoa has been evaluated as a new crop outside its original areas of cultivation (Van Schooten and Pinxterhuis, 2003; Jacobsen et al., 2005).

The plant's nutritional value is considerable and the whole plant has been used as animal feed (Galwey, 1989). Harvest residues are also used to feed cattle, sheep, pigs, horses and poultry (FAO, 1994). Rosero et al. (2010) indicated that a high proportion of Colombian livestock farmers know the quinoa crop, but a low proportion of farmers (20%) used quinoa in animal feed. Research in Denmark showed that quinoa could be a valuable forage crop for dairy farms when ensiled, with good yields and high protein content (Darwinkel and Stolen, 1998).

Although claims of a nutritionally favourable quality of quinoa seed have been made, only limited information is available on the evolution of the chemical composition in the whole plant at different growth stages. The objective of this study was to determine the fatty acid profile and nutritive value of quinoa seeds and plants during growth.

2. Materials and methods

2.1. Plant material

The quinoa seeds (Ayni variety), which was grown in Mantaro Valley (Peru), were kindly furnished by Dr. Aurelio Ciancio (Institute for Plant Protection, National Research Council, Bari, Italy), who obtained them from the Escuela de Nutrición Psicosomática (Lima, Peru). The study was conducted in the Western Po Valley near Cuneo, Italy. The quinoa stands were seeded in May 2010. No irrigation or fertilisers were applied after sowing. The herbage samples were collected from 1 m^2 subplots randomly located in 3 x 6 m^2 plots with three replicates. Plants were cut to a 1-2 cm stubble height. Sampling was performed in the morning after the evaporation of dew and was never carried out on rainy days. Herbage samples were collected with edging shears (0.1m cutting width) at six progressive morphological stages from early vegetative to grain fill stage from the end of June to the end of September 2010.

2.2. Chemical analysis

The herbage samples were immediately dried in a forced-draft air oven to a constant weight and the drying temperature was set at 65°C. The samples were then brought to air temperature, weighed, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1 mm screen and stored for qualitative analyses.

Whole seed and dried herbage samples were analysed by methods of AOAC (1990) for DM (#925.40), N (#984.13), and ash (#923.03). Neutral detergent fibre (NDFom), acid detergent fibre (ADFom) and lignin (sa) were determined with the Ankom^{200} Fibre Analyser (Ankom Technology Corp., Fairport, NY, USA), following the Ankom Technology Method and corrected for residual ash content. The NDF of herbage samples was analyzed without sodium sulfite and α-amylase. The gross energy (GE) was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

Fresh samples (200 g) of the herbage were refrigerated, freeze-dried and ground to pass through a 1 mm screen. Lipid extraction was performed on freeze-dried samples according to Hara and Radin (1978), while the transesterification of the FAs was performed according to Christie (1982), with the modifications described by Chouinard et al. (1999).

The FAs were analysed as their methyl esters. The analysis was carried out by gas chromatography, using a Dani GC 1000 DPC (Dani Instruments S.P.A., Cologno Monzese, Italy), equipped with a Supelcowax-10 fused silica capillary column (60 m x 0.32 mm (i.d.), 0.25 µm). The injector and detector ports were set at 245°C and 270°C, respectively. The oven temperature program was initially set at 50°C for the first min, and then increased at a rate of 15°C/min to 200°C, where it remained for 20 min and then increased at a rate of 5°C/min to 230°C, where it remained for the last 3 min. The carrier gas was helium. One microlitre was injected using a Dani ALS 1000 autosampler with a 1:50 split ratio. The peak area was measured using a Dani Data

Station DDS 1000, where each peak was identified and quantified according to pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

2.3 In vitro digestibility

The samples were also analysed to determine their *in vitro* dry matter digestibility (IVDMD) and NDF digestibility (IVNDFD) using the Daisy^{II} Incubator (Ankom Technology Corp., Fairport, NY, USA) according to Robinson et al. (1999). The *in vitro* rumen incubations were performed in two fermentative runs with different rumen inoculum. Ground samples (250 mg) were inserted into filter bags (Ankom F57 bags) which were then sealed. Jars were divided vertically by using a perforated plastic separator and 2 bags for each sample were inserted on either side of the separator, giving a total of 18 bags per jars. Moreover, seed samples were incubated in another jar in the same run. Digestion jars were filled with pre-warmed (39°C) buffer solutions and placed into the incubator. Rumen liquor was collected from rumen contents obtained at a slaughterhouse from cattle (two animals per run) of the same farm and fed a fibre-rich diet (Spanghero et al., 2010). Rumen liquor was filtered (through two layers of cheesecloth) and 400 ml of it was introduced into each jar together with the filter bags. After 48 h of incubation, the bags were removed, rinsed thoroughly with cold tap water and immediately analysed for NDF content with the Ankom^{200} Fibre Analyzer and incinerated to correct the residual NDF for the residual ash.

IVDMD was calculated using the following equation:

$$1-(W_3-(W_1*C_1))*1000/(W_2*DM),$$

where W_1 is the filter bag weight, W_2 is the sample weight, W_3 is the final weight (filter bag+residue) after in vitro and sequential treatment with NDF solution, C_1 is a comparison of the blank filter bag weight after and before digestion treatment and DM is the dry matter content (g/kg) of the samples.

IVNDFD was calculated using the following equation:

$$1-(W_3-(W_1*C_1))*1000/(W_2*NDF)$$

where W_1 is the filter bag weight, W_2 is the sample weight, W_3 is the final weight (filter bag+residue) after in vitro and sequential treatment with NDF solution, C_1 is a comparison of the blank filter bag weight after and before digestion treatment and NDF is neutral detergent fibre content (g/kg) of the sample.

2.4. Statistical analysis

The variability in the FA and herbage quality characteristics harvested at six different stages of maturity were analysed for their statistical significance via analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS Inc., 2002) to test the effect of the growth stage. In addition, single degree-of-freedom orthogonal contrasts were used to test for linear, quadratic and cubic effects due to morphological stage (Steel and Torrie, 1980). The effect on digestibility was tested based on a multiple linear regression with DM, ADF, NDF, and ADL content.

3. Results

3.1. Chemical composition

The proximate composition of the quinoa seed and plant during growth are presented in Table 2.

Quinoa seeds are higher in DM, OM, CP and GE contents than the plant during the growth cycle, while the lipid content was from twofold to threefold greater in the seed than in the plant during the growth cycle.

The results showed that the DM content increased linearly ($P<0.001$) and non-linearly ($P<0.01$) with advancing morphological stages from 130 g/kg fresh matter (FM), at the mid vegetative stage, to 189 g/kg FM at the grain fill stage. The CP content decreased quadratically or cubically ($P<0.001$) as the plant matured from 133 g/kg DM, at the early vegetative stage, to 94

g/kg DM at the budding stage, while the highest CP content, 151 g/kg DM, was recorded in the last stage (grain fill stage). At this stage, there is, in fact, protein accumulation in the seed. The NDFom and ADFom contents varied widely with maturity stage, ranged from 446 to 534 g/kg DM and from 219 to 370 g/kg DM, respectively. The linear increase in NDFom ($P<0.001$), ADFom ($P<0.001$) and lignin (sa) ($P<0.05$) amounts with increased stage of maturity is due to the progressive translocation of the soluble cell contents from the stems and leaves to the seed. The GE content decreased ($P<0.001$) linearly and non-linearly from the early vegetative stage to the mid vegetative stage and then increased from the late vegetative stage to the grain fill stage.

The pattern of FAs in the seed (Table 1) was characterised by three dominant FAs: palmitic acid (PA, $C_{16:0}$), OA and LA, representing about 823 g/kg of total FA.

The FA profile in the plant during growth (Table 3) is different from that of the seed oil. The whole plant was characterised by a high percentage of α-linolenic acid (ALA, $C_{18:3\ n-3}$) and of polyunsaturated fatty acids (PUFA), which made up from 385 to 474 g/kg of total FA and from 611 to 691 g/kg of total FA, respectively. LA content, which ranged from 146 to 176 g/kg of total FA, decreased ($P<0.05$) with increasing growth stages until the shooting stage and then increased, while other FAs did not show significant differences in their content during growth. Although there was difference for LA content among means, there were not differences explained by polynomial orthogonal contrasts for minor FAs. The average values of saturated FAs ($C_{14:0} + C_{16:0} + C_{18:0}$) ranged from 155 g/kg of the total FA at the late vegetative stage to 131 g/kg of total FA at the grain fill stage. Other non-identified FAs were detected in amounts that ranged from 77 g/kg of the total FA in the early vegetative stage to 147 g/kg of the total FA at shooting.

3.2. In vitro digestibility

The IVDMD and IVNDFD are presented in Table 2. Estimated digestibility at 48 hours of incubation showed significant linear decreases ($P<0.001$) with increasing stages of plant maturity.

Generally, at the grain fill stage, quinoa is less digestible. The IVDMD ranged from 0.921 g/g DM, at the early vegetative stage, to 0.714 g/g DM at the grain fill stage. Similarly IVNDFD showed significant linear decreases ($P<0.001$) with plant ageing and ranged from 0.837 to 0.429 g/g NDF. IVDMD depends on DM, NDF and ADF content (R^2=0.98, 0.98, 0.96 respectively). IVNDFD depends on DM and ADF content (R^2=0.98 and 0.97). There were not relationships between digestibilities and lignin content.

4. Discussion

The quinoa seed oil analysed in the present investigation has a lower LA content and higher stearic acid (SA, $C_{18:0}$) than those found by other authors and these differences can be ascribed to genetic variability. Quinoa oil appears to be a high-quality edible oil, similar in FA composition to soybean oil. Ruales and Nair (1993) found that quinoa fat had a high content of OA (245-248 g/kg of total FA) and LA (523 g/kg of total FA) and a low content of ALA (38-39 g/kg of total FA). The very high digestibility of quinoa seeds together with their attractive nutritive value raised their potential use as source of new grain, mainly in human nutrition against hunger (Miranda et al., 2012), but also in animal nutrition. In fact the outstanding nutritional and functional properties of quinoa seeds will be promoted by the FAO in 2013 since the United Nations has declared that year as the international year of quinoa.

Wood et al. (1993) reported that 11% of the total FAs of quinoa were saturated, with PA predominant, while LA, OA, and ALA accounted for 523, 230, and 81g/kg of total FA, respectively. Przybylski et al. (1994) found LA as the principal FA (560 g/kg of total FA) in quinoa, followed by OA (211 g/kg of total FA), PA (96 g/kg of total FA), and ALA (67 g/kg of total FA). Jahaniaval et al. (2000) reported the highest content of FAs from quinoa seed being 528 g/kg of total FA for LA and 70 g/kg of total FA for ALA, respectively.

As far as GE content of the quinoa seed is concerned, this value is lower than the GE of the other seed such as false flax (28.1 MJ/kg DM; Peiretti and Meineri, 2007), chia (26.1 MJ/kg DM; Peiretti and Gai, 2009) and *Galega officinalis* (20.5 MJ/kg DM; Peiretti and Gai, 2006).

Ramos and Cruz (2002) evaluated the performance of quinoa for forage production to obtain high quality feed for livestock in Cuba during the dry season and found DM of 18.9%, CP of 23.8% and crude fibre of 26.3%. They concluded that quinoa can improve the biological quality of feed for livestock.

Solíz-Guerrero et al. (2002) found that there was no difference in CP content at the panicle and blooming stages between a variety of soil water deficit treatments, but that it decreased during plant development.

Quinoa whole crop silage was used in a feeding experiment with dairy cows, comparing three rations: 35% of DM in the form of maize silage, 65, 45 or 25% wilted grass/clover silage and 0, 20 or 40% quinoa silage, respectively (Zom et al., 2002). This experiment showed that the DM intake of the ration with 20% quinoa silage was higher than with no or 40% quinoa silage, but due to the lower feeding values of the quinoa silage (57% digestibility of organic matter and a CP content of 80-100 g/kg DM) compared to the grass/clover silage, milk production and milk fat and protein content decreased with increasing quinoa silage in the ration, even though it was only for 40% quinoa silage that the decrease became significant.

As far as FA profile is concerned, the presence of ALA in the whole plant was at lower levels than those found in other forage crops such as *Galega officinalis* (Peiretti and Gai, 2006), false flax (Peiretti and Meineri, 2007), and flax (Peiretti and Meineri, 2008) at similar growth stages.

The digestibility of quinoa, determined using the Daisy *in vitro* technique, declines with advancing maturity during the growing season. Moreover, results show that NDF digestibility is one of the most variable quinoa parameters analysed, ranging from 43% for mature plants to 84% at the

early vegetative stage. Digestibility variability is greatly affected by the high proportion of DM and NDF. In fact, the differences in digestibility during plant maturity are primarily associated with the chemical composition of the samples, especially with their cell wall content and cell wall fractions. With advancing maturity, like all plants, quinoa develops xylem tissue for water transport, and accumulates cellulose and other complex carbohydrates; these tissues then become bound together by lignification (Hoffman et al., 2005). Lignification of plant tissues imposes a barrier to complete cell wall polysaccharide digestion in the rumen. For this reason, lignification of the plant cell wall is considered to be the primary impediment to forage digestibility (Jung et al., 2012). Since NDF digestibility influences animal performance (Oba and Allen, 1999), the optimal utilization of quinoa as forage for livestock must consider this parameter in order to assess forage quality and to rank it accurately. In general, our data confirm a high relationship between digestibility and DM, NDF and ADF contents of the plant during growth.

In conclusion, the first cut of quinoa, whose lipid fraction is rich in ALA and other polyunsaturated FAs, should be performed before shooting, since its nutritional quality deteriorates when cutting is delayed.

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