Chia \textit{(Salvia hispanica L.)} as a novel forage and feed source: A review

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Abstract

Chia \textit{(Salvia hispanica L.)}, is a traditional pre-Colombian food crop from Central America. Being considered the richest botanical source of omega-3 fatty acids, it has recently been rediscovered as a functional food and feed. A growing body of literature indicates that dietary chia seeds greatly improve animal products quality without compromising growth, productivity and organoleptic quality. Chia is mainly cultivated as a seed crop but recently interest has been raised on biomass production as a potential forage source opening alleys toward the integration of chia in crop-livestock systems. Literature on chia is flourishing, up to now reviews addressed botany, agronomy phytochemical and medicinal uses, this article reviews the main findings on chia use in animal nutrition and includes an overview on both seed and biomass yield and quality as affected by environment, agronomy, and genetic background. Chia is a short-day flowering crop, seed yields of commercial varieties can be as high as 2999 kg ha\textsuperscript{-1} in areas of origin while at European latitudes seed production is severely hampered by photoperiod sensitivity (max 518 kg ha\textsuperscript{-1}). The viable growing of chia for seeds worldwide relies on the availability of genotypes flowering at longer days than in the areas of origin, while for whole plant a relatively high forage yield can be expected. In southern Italy commercial short-day flowering varieties yielded up to 2.07 t ha\textsuperscript{-1} of leaf dry biomass and in Greece chia yielded up to 15 T ha\textsuperscript{-1} dry biomass, Chia seeds supplement in livestock diet are administered with the main objective to increase the content of omega-3 and improve animal health.

Introduction

\textit{Salvia hispanica L.} commonly known as Chia, Spanish sage or Mexican Salvia belongs to the genus \textit{Salvia} of the \textit{Lamiaceae} family. Chia originated at the low latitudes of Mexico and Guatemala, along with amaranthus, quinoa and maize it was one of the four staple foods of Mayas and Aztech populations (Munoz et al., 2013). After a long oblivion in recent years this crop has been rediscovered, nowadays it is cultivated as a seed crop and used as a functional food and feed. Chia is a source of proteins and secondary metabolites (Ayerza, 2013) among which antioxidants (Ayerza, 2013; Amato et al., 2015) but its popularity can be largely attributed to the exceptionally high content of alpha-linolenic acid (ALA), to the point of being considered one of the richest botanical source of omega-3 (Ayerza and Coates, 2001). Being a short-chain omega-3 fatty acid (FA), ALA is a precursor of long-chain (LC) omega-3, which has a major impact on health through many physiological mechanisms. They play a key role for the prevention and treatment of cardiovascular diseases (Nestel et al., 2015; Siscovick et al., 2017) and are very important for the development and maintenance of different organs, primarily the brain and nervous system. Emerging research demonstrated that they could be used as an adjuvant treatment for major depressive disorder (Mocking et al., 2016) as well as for reducing the risk of mood disorders (Berger et al., 2017). Increasing the dietary intake of omega-3 can contribute improve the omega-6:omega-3 ratio whose high values are associated with a greater incidence of cardiovascular disease, cancer, inflammatory and autoimmune diseases (Simopolous, 2008). Optimal values of this ratio should not exceed 4:1 but in western diet values around 20:1 are not uncommon (Simopolous, 2016). Many animal products are characterised by relatively high levels of saturated fatty acids, and omega-6/omega-3 ratio well above 4:1. According to Dalle Zotte and Szendro (2011) this ratio can be as high as 22 in pork loin and in chicken meat (16) while beef and rabbit meat have lower values (9...
and 7 respectively). Several researches proved the causal relationship between animal’s products such as red meat and increased incidence of cardiovascular disease, diabetes and some types of cancer (Pan et al., 2012; Abete et al., 2014). Egg industry over the last decade has been penalised by a negative trend in per capita egg consumption possibly linked to the fact that eggs are a major dietary sources of cholesterol and consumers are concerned about the correlation between cholesterol intake and cardiovascular disease (Zazpe et al., 2011). In order to improve the nutritional profile of animal products omega-3 feed enrichment has been extensively investigated in many sectors such as poultry science (Cherian and Sim, 1991; Gonzalez-Esquerra and Leeson, 2001; Fraye et al., 2012), rabbit nutrition (Bernardini et al., 1999; Dal Bosco et al., 2004; Kouba et al., 2008; Peiretti and Meineri, 2010; Dal Bosco et al., 2014) as well as pig nutrition (Morgan et al., 1992; Leskanich et al., 1997). In recent years special attention has also been paid by dairy industries to improve the nutraceutical profile of milk by increasing the content of polyunsaturated fatty acids (PUFA) omega-3 FA (Dewhurst et al., 2006). Ruminants cannot synthesise omega-3 endogenously so their content in milk depends on their proportion in ingested feed and is mediated by rumen bio-hydrogenation processes (Witkowska et al., 2008). Lipid profile in milk can be sharply modified by feeding animals with forages rich in omega-3 (Dewhurst et al., 2003) as well as by supplementing oilseeds or marine oils (Chilliard et al., 2001). In order to improve consumers acceptability industries developed innovative omega-3 fortified animal products that are fuelling an emerging but fast-growing market worldwide. A list of leading brands for omega-3 fortified animal products in Europe has been reported by Kolanowski and Laufenberg (2006). The necessity of finding botanical sources of omega-3 has even been raised in aquaculture where farmers need to find innovative plant-based feeds as an alternative to overexploited marine sources. According Sprague et al., (2016) in Norwegian salmon farming industry the recent shift from marine to plant-based feeds, with rapeseed oil being one of the most common alternatives to fish oil, has resulted in higher omega-6 levels in fish tissue causing an increase of omega-6:omega-3 ratio with consequences on fish nutritional quality. While in this sector much emphasis is placed on the necessity of finding alternative (to marine sources) dietary sources of LC omega-3, salmonids and many freshwater species have complete metabolic pathways to produce LC omega-3 from ALA (Tocher, 2015). Chia is raising so much interest as a nutraceutical food and feed, that books and reviews are appearing, addressing botanical and agronomic aspects (Ayerza and Coates, 2005; Bochicchio et al., 2015a); phytochemistry and pharmacological properties of the seeds (De Falco et al., 2017a), as well as nutritional, functional properties and therapeutic prospects (Valdivia-Lopez and Tecante, 2015; Ulla et al., 2016). There is a growing body of literature on chia seeds use in animal nutrition (Ayerza and Coates, 2000, 2002, 2005, 2006; Ayerza et al., 2002; Azcona et al., 2008; Peiretti and Meineri, 2008) and recently interest has been raised on biomass production as a potential forage source (Peiretti and Gai, 2009; Amato et al., 2015) opening new alleys toward the introduction of chia in forage systems. The aim of present article is to provide a comprehensive account of chia potential as a forage and feed; we therefore address chia use in animal nutrition along with a review of the published works on chia seed and biomass yield and of quality traits relevant to the food chain of animal products, as a function of agronomic management and genetic background. This may serve as a basis to evaluate the feasibility of introducing chia in crop-livestock systems and a short section on future prospects is included.

### Botany and history

Chia (*Salvia hispanica* L.) is an annual macrothermal crop that belongs to the *Lamiaceae* family. The centre of origin based on genetic and phenotypic diversity has been identified as the area from Western Mexico to Puebla, with main altitudes between 1400 and 2200 m a.s.l. (Cahill, 2004). It is generally 60-to-180-cm tall (Capitani et al., 2013), with opposite, petiolate, and serrated leaves; flowers are hermaphrodite and grow in numerous clusters in a spike, and preserved by small bracts with long pointed tips (Ayerza and Coates, 2005). Non-deshinct dry fruits are routinely named seeds (Capitani et al., 2013) and in domesticated chia mean seed mass is about 1.5 g/1000 seeds (Cahill and Ehdai, 2005) (Figure 1). Chia was a staple food of pre-Columbian Central America populations, and was even introduced in Spain after the conquest (Ortiz de Montellano, 1978), but it was dramatically eradicated for the subsequent 500 years following religious conflicts (Ayerza and Coates, 2005). In pre-Columbian Mesoamerica, chia was valued for food use of seeds, medicine and oil (Cahill, 2003), and in recent years several studies have indicated chia as a new oilseed plant due to its high ω-3 fatty acid content, probably the highest among other oilseeds (Cahill, 2003, 2004; Ayerza, 1995, 2011, Ayerza and Coates, 2004, 2007, 2009). Chia is nowadays mainly grown for seeds and commercialised as functional food. However the leaves have potential commercial interest due to their composition (Bushway, 1981) and content of active compounds of nutraceutical, antioxidant and antimicrobial value (Ahmed et al., 1994; Amato et al., 2015; Elshafie et al., 2018). In the last few decades pioneering work by Peiretti and Gai (2009) and Peiretti (2010) has shown the potential of chia seeds as feed and of chia whole plants as functional forage for their content of omega-3.

### Seed and biomass yield

Based on report of Alenbrant et al. (2014), the largest centre of production is Mexico from where seeds are exported to Japan, USA, and Europe. Chia is also grown in Argentina, Bolivia, Colombia, Guatemala, Peru, Australia, Africa, and Southeast Asia (Eplng, 1940; Perry and Metzger, 1988; Jansen et al., 1991). Research from Europe (Bochicchio et al., 2015a, 2015b) reports on seed and whole-plant yield.

In the nineties Ayerza and Coates started a study on potential production of chia as an alternative crop in several locations of Argentina and report data from commercial fields or research plots (Coates and Ayerza, 1996, 1998). A large variation in yields (175 to 1602 kg ha⁻¹) can be ascribed to different locations (with corresponding weather conditions) and agronomic practices, and the authors conclude that early sowing affects production greatly and allows to double yields (882 kg ha⁻¹ with early sowing vs 450 and 437 kg ha⁻¹ for sowings 27 and 46 days later) (Coates and Ayerza, 1996). A more recent study conducted in Ecuador (Ayerza and Coates, 2009) ascertained higher maximum yields (up to 2500 kg ha⁻¹) but still a large variation with location (and corresponding elevation). Out of three chia selections tested in the experiment, Itzac 1 was the most productive. In Ghana Yeboah et al. (2014) showed very high maximum yields (2999 kg ha⁻¹) with high sowing density of 40.000 plants ha⁻¹ and direct sowing, while lower yields were recorded with lower densities and transplanted seedlings.
Studies at high latitudes in Europe (Amato et al., 2015; Bochicchio et al., 2015b) and Chile (Silva et al., 2016) showed low yields (max 518 kg ha⁻¹) without a significant effect of nitrogen fertilisation (Amato et al., 2015; Bochicchio et al., 2015b) or irrigation (Silva et al., 2016) but with a positive effect of sowing density (Bochicchio et al., 2015b). This was linked to late flowering of chia at high latitudes (Bochicchio et al., 2015b): chia is a self-pollinated short-day flowering plant and the major limiting factor for expanding its cultivation from tropical-subtropical areas to other regions is the temperature and photoperiod sensitivity. At high latitude the crop cannot be sown before late spring and therefore long summer days will delay flowering until early fall and seed maturation will be hampered by low temperatures (Jamboonsri et al., 2012). New chia long-day flowering or photoperiod insensitive mutants were created by Jamboonsri et al. (2012) and some of them were characterised for their yield, agronomic and/or metabolic behaviour at higher latitudes than those of the area of origin (De Falco et al., 2018a, 2018b). A study comparing commercial short-day flowering chia with long-day flowering mutant G8 at high latitude showed low yield regardless of irrigation for the former (less than 300 kg ha⁻¹), whereas G8 yielded up to 2553 kg ha⁻¹ with a significant response to irrigation (De Falco et al., 2017b). A study stretching across latitudes in Chile (Baginsky et al., 2016) confirmed that high yields (up to 2903 kg ha⁻¹) of short-day flowering chia could be reached at low latitudes whereas lower yields (as low as 70 kg ha⁻¹) are obtained at high latitudes due to late flowering. Some of the studies on seed yield also report plant biomass values at flowering or maturity, and confirm the effect of location and agronomic practices on production with a positive effect of irrigation (Silva et al., 2016) and sowing density (Yeboah et al., 2014; Bochicchio et al., 2015b; Bilalis et al., 2016). Values range from 492 kg ha⁻¹ reported by Coates and Ayerza (1996) in Argentina to 15,357 kg ha⁻¹ reported by Bilalis et al. (2016) in Greece. A dry biomass of around 2070 kg ha⁻¹ of leaves was reported by Amato et al. (2015). It should be noted that late flowering of traditional chia selections at high latitudes might result in vegetative biomass accumulation due to a longer vegetative stage. Therefore a higher biomass and a lower harvest index is reported at higher latitude (Baginsky et al., 2016) in Chile and for commercial short-day flowering chia compared to the long-day flowering mutant G8 at high latitude in Southern Italy (De Falco et al., 2018b). A glasshouse pot study showed a positive effect of arbuscular mycorrhiza inoculation on chia fresh plant biomass (Ouzounidou et al., 2015).

Seed and biomass quality

According to USDA (2011) chia seeds show high oil content (30.4%) but also a remarkable amount of proteins, calcium, and other elements and vitamins (Table 1). Since its rediscovery in the nineties, research on nutrients and active compounds in chia seeds

| Nutrient                  | per 100 g |
|--------------------------|-----------|
| Energy (kcal.)           | 486.00    |
| Proteins (g)             | 16.54     |
| Total fat (g)            | 30.74     |
| Saturated fatty acids (g)| 3.33      |
| Monounsaturated fatty acids (g) | 2.31 |
| Polyunsaturated fatty acids (g) | 23.67 |
| Trans fatty acids (g)    | 0.14      |
| Omega-3 fatty acids (g)  | 17.83     |
| Cholesterol (mg)         | 0.00      |
| Carbohydrate (g)         | 42.12     |
| Fibre, total dietary (g) | 34.40     |

Source: U.S. Department of Agriculture (2011)

Figure 1. Left: Picture of the non-dehiscent chia dry fruits that are routinely named seeds, in domesticated chia seeds have and average length of 2 mm and a width of about 1.3 mm, a thousand seeds weights around 1.5 g. Right: frontal view of a chia stand in Southern Italy during vegetative stage, chia has opposite, petiolate, and serrated leaves; height can range between 60 and 180 cm tall, leaves production can reach 2 tons/ha.
has been conducted by many authors, but also the composition and more recently the forage quality of chia stems and leaves has been inquired, and seed composition has been found to be affected by factors such as genotype, environment, and agronomical inputs (Ayerza and Coates, 2004; Amato et al., 2015; De Falco et al., 2017b, 2018a, 2018b). Tables 2 and 3 report the main findings on seed and biomass yield as a function of environment, genotype and agronomic practices, Tables 4 and 5 report the current body of knowledge on seed and biomass quality respectively. In the following paragraph a review of available data on quality and yield will be reported for each seed and plant component.

Oil

Based on many reports, oil content of chia seeds range from 20.30 to 38.60% (Ayerza, 1995; Ixtaina et al., 2011; Ayerza and Coates, 2004; Da Silva Marinel et al., 2014; Amato et al., 2015). The main fatty acids of chia seed are linolenic acid (18:3), linoleic acid (18:2), stearic acid (18:0), palmitic acid (16:0) and oleic acid (18:1) (Ayerza, 1995; Coates and Ayerza, 1998; Ayerza and Coates, 2004; De Falco et al., 2018b). ALA constitutes the highest percentages (>60%) (Ayerza, 1995; Coates and Ayerza, 1996; Peiretti and Gai, 2009; Amato et al., 2015; De Falco et al., 2018b). This ALA content is above the percentages reported for other ALA-rich oilseed crops such as flax (Linum usitatissimum L.) (57%) (Ayerza and Coates, 2004), false flax (Camelina sativa L.) (48.4%) (Peiretti and Meineri, 2007), and similar or slightly lower to that of perilla (Perilla frutescens L.) (59.8% and 60.9% for chia and perilla respectively) (Ciftci et al., 2012) making chia one of the richest plant-based omega-3 source (Ayerza and Coates, 2001).

Furthermore, chia seeds are known as a good source of vitamins A and C and niacin (USDA, 2011). Ecosystem effects on oil content of chia have been reported. A wide range in oil content and fatty acid content of chia seeds grown under various climatic conditions, and in different geographical areas has been shown by Ayerza (1995), Coates and Ayerza (1996, 1998), and Ayerza and Coates (2004). Also oil content can be affected by extraction methods (Ixtaina et al., 2011). Genotype and environmental factors appear to affect the fatty acid composition more than the total oil content as reviewed by Bochicchio et al. (2015a). Specifically, low temperatures (and therefore elevation) positively affect the level of unsaturation of fatty acids in chia as for other oil seed crops (Ayerza, 2009; Ayerza and Coates, 2004, 2011), and therefore oil saturation and the ratio of n-6: n-3 fatty acids decreases with increasing altitude (Ayerza and Coates, 2011). Also, oil content

| Table 2. Seed yield. |
|----------------------|
| Production area       | Experimental factors | Yield     | Reference             |
| Northwestern Argentina (23°17′ S to 28°35′ S) | Seeding date and locations | 175 to 1602 kg ha⁻¹ | Coates and Ayerza, 1996 |
| Northwestern Argentina (24°23′ S to 25°03′ S) | Geographic locations | 221 to 1262 kg ha⁻¹ | Coates and Ayerza, 1998 |
| Ecuador (01°18′00″ S, 00°03′26″ S, and 00°29′47″ N) | Location and chia selections | 295 to 2300 kg ha⁻¹ | Ayerza and Coates, 2009 |
| Ghana, Kumasi (06°43′ N, 01°36′ W) | Planting methods and planting density | 1754 to 2999 kg ha⁻¹ | Yebobah et al., 2014 |
| Southern Italy (40°51′37.59″ N, 15°38′49.43″ E) | Fertilisation | 182 to 269 kg ha⁻¹ | Amato et al., 2015 |
| Southern Italy (40°51′37.59″ N, 15°38′49.43″ E) | Sowing density × fertilisation | 134 to 516 kg ha⁻¹ | Bochicchio et al., 2015a, 2015b |
| Chile (18°30′ S to 33°30′ S) | Sowing dates and 2 chia phenotypes | 70 to 2903 kg ha⁻¹ | Baginsky et al., 2016 |
| Northern Chile (30°02′16″ S, 70°41′48″ W) | Irrigation × phenotype | 218 to 381 kg ha⁻¹ | Silva et al., 2015 |
| Southern Italy (40°51′37.59″ N, 15°38′49.43″ E) | Commercial back chia vs short-day flowering mutant G8 × irrigation regime | 110 to 2553 kg ha⁻¹ | De Falco et al., 2018a |

| Table 3. Biomass yield. |
|-------------------------|
| Plant part | Production area | Experimental factors | Yield | Reference |
| Whole plant mass | Northwestern Argentina (23°17′ S to 28°35′ S) | Seeding date and locations | 492 to 3675 kg ha⁻¹ | Coates and Ayerza, 1996, 1998 |
| Whole plant mass | Northwestern Argentina (24°23′ S to 25°03′ S) | Geographic locations | 1125 to 4178 kg ha⁻¹ | Yebobah et al., 2014 |
| Ghana, Kumasi (06°43′ N, 01°36′ W) | Planting methods and planting density | 1081 to 2119 kg ha⁻¹ | Coates and Ayerza, 1998 |
| Attikis, Greece glasshouse | Soil pH and arbuscular mycorrhiza inoculation | 23 to 40.2 g plant⁻¹ | Ouzounidou et al., 2015 |
| Southern Italy (40°51′37.59″ N, 15°38′49.43″ E) | Sowing density × fertilisation | 50.87 to 59.71 t ha⁻¹ | Bochicchio et al., 2015a, 2015b |
| Northern Chile (30°02′16″ S, 70°41′48″ W) | Irrigation × phenotype | 1830 to 3491 kg ha⁻¹ | Silva et al., 2016 |
| Greece (37°59′12″ N, 23°42′96″ E) | Sowing rates and organic fertilisation | 4484 to 15,357 kg ha⁻¹ | Bilalis et al., 2016 |
| Chile (18°30′ S to 33°30′ S) | Sowing dates and 2 chia phenotypes | 1854 to 12,415 kg ha⁻¹ | Baginsky et al., 2016 |
| Southern Italy (40°51′46.80″ N) | Commercial chia and long-day flowering mutants | 33.06 to 54.28 g/plant | De Falco et al., 2018b |

| Leaf biomass | Southern Italy (40°51′37.59″ N, 15°38′49.43″ E) | Sowing density × fertilisation | 11 t ha⁻¹ fresh biomass | Bochicchio et al., 2015a, 2015b |
| Leaf biomass | Southern Italy (40°51′37.59″ N, 15°38′49.43″ E) | Fertilisation | 10.8 t ha⁻¹ fresh biomass, 2.07 dry biomass | Amato et al., 2015 |
Table 4. Seed quality.

| Production area | Experimental factors | Quality traits | Reference |
|-----------------|----------------------|----------------|-----------|
| Northwestern Argentina (23°20’ S to 28°23’ S) | Location | Fatty acids composition | Añezza, 1995 |
| Northwestern Argentina (23°17’ S to 28°35’ S) | Seeding date and locations | Fatty acids composition | Coates and Añezza, 1996 |
| Northwestern Argentina (24°23’ S to 25°03’ S) | Geographic locations | Oil content and fatty acids composition | Coates and Añezza, 1998 |
| Glasshouse Israel | Irrigation water | Oil content and fatty acids composition | Hueser et al., 2002 |
| America (4°31’ S to 28°28’ S) | Location | Oil and protein content, peroxide index, fatty acids composition | Añezza and Coates, 2004 |
| Commercial source | Location | Soluble and insoluble fibres, phenolic compounds, antioxidant activity | Reyes-Caudillo et al., 2008 |
| Commercial source | Fatty acids composition | Peiretti and Gai, 2009 |
| Argentina, Bolivia, Ecuador, Tropical rain forest, Inter-Andean Dry Valley ecosystem (62°18’00” S to 25°07’45” S, 00°29’47” N to 00°45’00” N) | Location | Oil and protein content, fatty acids composition | Añezza, 2009 |
| Ecuador (01°18’50” S, 00°03’26” S, and 00°29’47” N) | Location and chia selections | Oil and protein content, fatty acids composition | Añezza and Coates, 2009 |
| Ecuador (00°03’26” S to 2°18’00” S, 00°47’50” N to 01°47’50” N) | Phenotype (seed coat colour) and growing locations | Oil content and fatty acids composition | Añezza, 2010 |
| Argentina and Guatemala | Two oil extraction methods | Oil content and quality: fatty acids composition, colour, carotenoids, chlorophyll, metals, tocopherols, phytosterols, oxidative stability rancimat | Ixtaina et al., 2011 |
| America (25°07’48” S), Bolivia (17°17’00” S to 17°24’00” S) and Ecuador (01°18’50” S to 00°29’47” N) | Location | Oil and protein content, fatty acids composition | Añezza and Coates, 2011 |
| Ecuador (Tropical Forest ecosystem) | Chia seed phenotype (seed coat colour) and its composition | Oil and protein content, water %, peroxide value, soluble and insoluble fibre, fatty acids composition, aminoaids concentration, antioxidant compounds (flavonols and lignans) | Añezza, 2013 |
| Commercial source | Black chia seed | Oil and protein content, water %, ash, total fibre, oil peroxide and iodine index, fatty acids tocopherols, phytosterols | Alonso-Calderón et al., 2013 |
| Commercial source | Chia seed | Oil and protein content, water %, peroxide value, thiobarbituric acid reactive substances, DPPH free radical scavenging activity, ferric reducing antioxidant power, oxygen radical absorbance capacity, soluble and insoluble fibre, fatty acids composition, antioxidant compounds (polyphenols) | Da Silva Marinel et al., 2014 |
| Colima, Mexico | Chia seed | Phenolics and isoflavones content, DPPH (2,2-diphenyl-1-picyrylhydrazyl) assay | Martinez-Cruz and Paredes-López, 2014 |
| Southern Italy (40°51’37.59” N, 15°38’49.43” E) | Seed source, fertilisation | Water and oil content, fatty acids composition, free acidity, peroxide index, p-anisidine value, chlorophyll, carotenoids, tocopherols, phenolics, antioxidant activity Trolox equivalent, oxidative stability Oxitest | Amato et al., 2015 |
| Chile (18°30’ S to 33°30’ S) | Sowing dates and 2 chia phenotypes | Oil content and fatty acids profile | Bagijsky et al., 2016 |
| Northern Chile (30°02’16” S, 70°41’48” W) | Irrigation × phenotype | Oil content, linoleic and alpha-linoleic fatty acids content | Silva et al., 2016 |
| Southern Italy (40°51’37.59” N, 15°38’49.43” E) | Chia populations and long-day flowering mutants, nitrogen fertilisation | Metabolomic analysis: fatty acids, sugars, caffeoyl derivatives, flavonoids, organic acids, free amino acids | De Falco et al., 2017b |
| Southern Italy (40°51’37.59” N, 15°38’49.43” E) | Commercial black chia vs long-day flowering mutant G8 × irrigation regime | Metabolomic analysis, fatty acids, sugars, caffeoyl derivatives, flavonoids, organic acids, free amino acids, phenolics, antioxidant activity Trolox equivalent | De Falco et al., 2018a |
and fatty acids composition are affected by chia selection (Ayerza and Coates, 2009), sowing date (Coates and Ayerza, 1996; Baginsky, 2016), and salinity of irrigation water (Heuer et al., 2002). No difference was found in oil content and composition between chia phenotypes by Ayerza (2010), but Silva et al. (2016) found a higher content of omega-3 in white chia compared to the black phenotype, and De Falco et al. (2018b) found a higher content of omega-3 in a white chia phenotype compared to black chia and long-day flowering mutants. Also, De Falco et al. (2018a) found a higher content of omega 3 in the long-day flowering mutant G8 compared to a commercial black chia, both grown in southern Italy. Irrigation was not found to affect oil content by Silva et al. (2016) and De Falco et al. (2018a), but it changed the composition of fatty acids and increased omega-3 and reduced the oleic/linoleic ratio from 47.4 in rainfed plots to 39.6 in irrigated plots. Peiretti and Gai (2009) investigated vegetative parts of chia and found a content of fats decreasing from 30 g kg–1 at the early vegetative stage to 18 g kg–1 at the budding stage. The percentage of polyunsaturated fatty acids was high and decreased from 752 g kg–1 to 623 g kg–1 of the total plant fatty acids during plant growth. The most abundant fatty acid was alpha-linolenic acid, but its proportion to other fatty acids varied from a maximum of 649 g kg–1 at early vegetative and 499 g kg–1 at budding. According to Ouzounidou et al. (2015) fat content and composition in chia leaves is affected by soil pH and inoculation with arbuscular mycorrhiza.

### Table 5. Biomass quality.

| Plant part           | Production area                      | Experimental factors                          | Quality traits                                                                 | Reference          |
|----------------------|--------------------------------------|-----------------------------------------------|-------------------------------------------------------------------------------|--------------------|
| Whole plant          | Karad, India                         | Different levels of ethanolic extracts of chia| Anthelmintic efficacy                                                          | Patil et al., 2014 |
| Stem and leaves      | Po valley, Northern Italy            | Plant growth stage                             | Fatty acids composition, dry matter, organic matter, crude protein, ether extract, ash, acid detergent fibre and neutral detergent fibre, lignin, in vitro organic matter digestibility, gross energy | Peiretti and Gai, 2009 |
| Stem and leaves      | Po valley, Northern Italy            | Herbage and silage according to plant growth stage | Herbage composition and ensilability characteristics: dry matter, water soluble carbohydrates content, pH, buffering capacity, soluble and total nitrogen, gross energy, alcohol, volatile fatty acids, lactic acid | Peiretti, 2010     |
| Stem and leaves      | Greece (37°39′12″ N, 23°42′56″ E)     | Sowing rates and organic fertilisation         | Dry matter, organic matter, crude protein, ether extract, ash, acid detergent fibre and neutral detergent fibre | Bilalis et al., 2016 |
| Stem and leaves      | Italy, Southern Italy (40°51′37″59′′ N, 15°38′49″43′′ E) | Long-day flowering mutant genotype (G8)       | Composition of essential oils, fungicidal and bactericidal assays               | Elshafie et al., 2018 |
| Leaves               | Southern California, South-eastern Texas, and Northwestern Argentina | Location                                      | Composition of essential oils                                                | Ahmed et al., 1994 |
| Leaves               | Southern Italy (40°51′37″59′′ N, 15°38′49″43′′ E) | Fertilisation                                  | Metabolomics of methanol extract: flavonoids and hydroxycinnamic acid derivatives | Amato et al., 2015 |
| Stems, leaves and roots | Attikis, Greece                | Soil pH and arbuscular mycorrhiza inoculation | Oil, protein and carbohydrate content, fatty acids composition and phenolics of plant shoots, content of P in roots and shoots | Ouzounidou et al., 2015 |

### Protein

According to Ayerza and Coates (2004, 2009, 2001) chia seeds have high levels of protein (16-26%, depending on environment), compared to cereal seeds or oilseds, even though chia is not commercially grown as a protein source around the World. Values will also change under different agronomic, climatic and soil conditions (Ting et al., 1990). Ayerza and Coates (2004) report a variation in protein content of chia seeds from locations in four different countries but could not totally explain it with environmental factors. Chia seed protein has nine essential amino acids (Ayerza, 2013), with more balanced composition in comparison with other grains (Ayerza and Coates, 2001) especially with regard to cysteine and methionine (Ixtaina et al., 2008; Sandoval-Oliveros and Paredes-López, 2013). Crude protein content in vegetative parts of the plant have been studied by Peiretti and Gai (2009) and Peiretti (2010), who reported values decreasing from 188 g kg–1 at the early vegetative stage to 57 g kg–1 at budding. According to Bilalis et al. (2016) crude protein in chia biomass increases with manure fertilisation vs other organic fertilisers, and with narrow row spacing. Ouzounidou et al. (2015) report a variation in plant protein content according to soil pH and inoculation with arbuscular mycorrhiza.

### Fibre

Recent studies have shown that total dietary fibre content in the chia seed ranges between 32.4 and 37.50 g/100 g, most of
which is insoluble (>93%) and the rest soluble (<7%) (Reyes-Caudillo, 2008; da Silva Marineli et al., 2014). Chia meal has a high level of dietary fibre (33.9-39.9% of dietary fibre per 100 g) according to Capitani et al. (2012), and therefore it has a high potential for human and animal nutrition (Ting et al., 1990). Numerous studies have shown the effect of potential health benefit of chia seed fibre consumption in some disease such as coronary heart disease, risk for diabetes type 2, and cancer (Steinmetz and Potter, 1996; Lattimer et al., 2010; Kaczmarczyk et al., 2012). Part of the chia dietary fibre is located in the epidermal cells of the seed and it swells when hydrated (Valdivia-López and Tecante, 2015), forming a mucilage capsule around the seed. This mucilage may be extracted (Muñoz et al., 2012). The content of surface mucilage is 5 to 10% (Muñoz et al., 2012; Reyes-Caudillo et al., 2008; Ayerza and Coates, 2001). Health benefits of chia seed mucilage are reviewed by De Falco et al. (2017a) and are mainly related to the control of health in the digestive system and obesity, with related coronary disease. The mucilage extracted from chia seeds has an excellent potential in food technology as a thickener (Munoz et al., 2012), it can be used as a basis for films to avoid food dehydration (Capitani et al., 2015, 2012; Muñoz et al., 2012), as a replacement for fat in cakes (Felisberto et al., 2015), and as an additive to improve rheology and nutraceutical properties of gluten-free pasta (Menga et al., 2017). Rheological and physico-chemical effects of the mucilage in soil have also been explored by Di Marsico et al. (2018a, 2018b), who showed a dose- and soil-dependent significant increase in soil aggregate stability and changes in soil porosity and herbicide-soil interactions.

Antioxidants and other compounds

Besides fatty acids, In addition to fatty acids composition, protein and fibre, chia seed has other important ingredients as natural antioxidants, which have beneficial influence on human health (Nijveldt et al., 2001). These compounds have been reviewed by De Falco et al. (2017a) and include phenolic contents such as chlorogenic acid, caffeic acid, myricetin, quercetin, kaempferol and 3, 4-dihydroxyphenylethanol-enolic acid diol diethylene (3, 4-DHPEA-EDA), and tocopherols, phytosterols, lignans and carotenoids (Reyes-Caudillo et al., 2008; Ayerza, 2013; Alonso-Caldérón et al., 2013; Martínez-Cruz and Paredes-López, 2014; Da Silva Marineli et al., 2014; Amato et al., 2015; De Falco et al., 2017b, 2018a, 2018b). Health benefits of such compounds have been identified (Ayerza and Coates, 2005; Vuksan et al., 2007) and reviewed by De Falco et al. (2017a). No evidence of allergic and toxic effects upon consumption has been reported (EFSA, 2009). Several authors report a rather high antioxidant capacity or oxidative stability of chia seeds (Reyes-Caudillo et al., 2008; Ixtaina et al., 2011; Coelho and de las Mercedes Salas-Mellado, 2014; Da Silva Marineli et al., 2014; Martínez-Cruz and Paredes-López, 2014; Amato et al., 2015; De Falco et al., 2018a, 2018b). According to Amato et al. (2015) fertilisation with inorganic N resulted in an increase of free acidity, chlorophyll and carotenoids, whereas it reduced p-anisidine value, phenols and oxidative stability. A series of metabolomic studies of chia seeds comparing traditional sources such as commercial black and white chia with long-day flowering mutants were performed by De Falco et al. (2017b, 2018a, 2018b). They report that apolar organic extracts were mainly composed of mono- and polyunsaturated fatty acids and polar organic extracts contained sugars such as glucose, raffinose, sucrose, methylgalactoside as well as caffeoyl derivatives, flavonoids, organic acids, free amino acids. De Falco et al. (2017b) reported Tashimone 1 and 15, 16 dihy-dro Tanshinone 1 in chia seeds for the first time. The metabolic fingerprinting of different chia sources showed that black phenotypes are richest in carbohydrates, white chia in omega-3 (De Falco et al., 2017b) and according to De Falco et al. (2018b) long-day flowering mutants G17 and G8 are rich in nutraceuticals (such as quinic acids, caffeic acid and others). According to De Falco et al. (2018a) mineral nitrogen top-dressing increased the content of aliphatic free amino acids, and decreased the level of carbohydrates and flavonoids, but not cafefol derivatives, organic and fatty acids. They also report a higher content in polyphenols in long-day flowering mutants compared to commercial black and white chia, and this was related with the level of total polyphenols but not with the content of other antioxidants such as malic, citric and quinic acids. Ahamed et al. (1994) identified fifty-two different compounds in chia leaves, and report that the major component detected in essential oils was P-caryophyllene. Elshaffie et al. (2018) found more than eighty compounds in essential oils from vegetative parts of chia with caryophyllenes as the main components and 1.5% of phenolic compounds. Amato et al. (2015) identified thirty-four compounds in methanol extracts of chia vegetative parts. Among them several flavonoids and hydroxycinnamic acids. They found two uncommon flavonoids (acetyl vitexin and acetyl orientin) not previously reported in Lamiaceae.

Minerals and vitamins

Chia seed besides other main ingredients is a great source of some nutritional components like minerals and vitamins. So that, early researches are related to content of vitamin B in seeds (Bushway et al., 1984). Chia fruits have a high level of vitamin B (Bushway et al., 1984) compared with most other cereals. Also it is excellent source for minerals as calcium, phosphorus, potassium, zinc and copper (Ayerza, 2001). According to recent evidence, macronutrients concentration of chia seeds are phosphorus 860, calcium 631, potassium 407, and magnesium 355 mg/100 gm, and microelements; selenium 55.2, sodium 16, iron 7.72, manganese 2.72, zinc 4.58, copper 0.924, molybdenum 0.2 μg/100 g (Ullah et al., 2016). Phosphorous, calcium and potassium content of chia seed is more than other crops such as wheat, rice, oats and corn (Beltran-Orozco and Romero, 2003).

Chia in animal nutrition

The use of chia in animal nutrition until now has been limited to the use of seeds, either raw or processed (grinding/pelleting) and oil, and to the use of by-products such as discarded seeds and seed meal. The main objective has been increasing the content of omega-3 fatty acids of animal products. So far chia dietary use has been tested on monogastric (rabbits, pigs, broilers, hens and quails), on ruminants (on dairy cows and goats, and for lamb fattening), on fishes (freshwater aquaculture) and recently on edible insects (Table 6). As mentioned above chia is mainly grown as a seed crop, recently though whole plant or leaf biomass has been proposed as forage, because of useful and health-promoting compounds in vegetative tissues (Ahmed et al., 1994; Peiretti and Gai, 2009; Peiretti, 2010; Amato et al., 2015). There are no reports on the effect of whole plant use in animal diets. Therefore the following paragraphs will report the key findings of the published feeding trials followed by a section on the perspective forage use and a brief overview of other potential uses in livestock systems.

Chia use in poultry science

As shown in Table 6 the largest number of studies on chia use
Table 6. Feeding trials.

| Animal         | Feed ingredient | Inclusion rate (w/w) | Experiment duration | Evaluated parameters                                                                 | Reference                        |
|----------------|-----------------|----------------------|---------------------|--------------------------------------------------------------------------------------|----------------------------------|
| **Monogastrics** |                 |                      |                     |                                                                                      |                                  |
| **Poultry**    |                 |                      |                     |                                                                                      |                                  |
| Laying hens    | Whole seed      | 0, 30%               | 4 weeks             | Egg yield, FA profile, cholesterol content, organoleptic profile                     | Ayerza and Coates, 1998          |
|                | Whole seed      | 0, 7, 14, 21, 28%    | 90 days             | Egg yield, cholesterol content, eggs FA profile                                      | Ayerza and Coates, 2000          |
|                | Whole seed      | 0, 7, 14, 21, 28%    | 30 days             | Egg yield, FA profile, cholesterol content, organoleptic profile                     | Ayerza and Coates, 2001          |
|                | Whole seed      | 0, 7, 14, 21, 28%    | 90 days             | Hen weight, egg production, egg weight, and yolk weight and percentage                | Ayerza and Coates, 2002          |
|                | Whole seeds     | 0, 7, 15, 0%         | 16 weeks            | Productive performance and lipid composition of the egg yolk and body tissues        | Salazar-Vega et al., 2009        |
|                | Whole seed and chia oil | 0, 25% chia seed, 0% oil | 84 days            | Production performance, egg yolk, total fat and fatty acid composition             | Astruejo et al., 2011            |
|                | Whole seed      | 0, 20, 30, 40%       | 5 weeks             | FA composition, sensory attributes                                                  | Coorey et al., 2015              |
|                | Whole seed      | 0, 5, 75%            | 4 weeks             | Egg fatty acid profile, short to long-chain omega 3 FA bioconversion efficiency      | Komprda et al., 2013             |
| Broiler        | Whole seed      | 0, 10, 20%           | 7 weeks             | White and dark meat fatty acid profile, broilers productive performance, sensory attributes | Ayerza et al., 2002              |
|                | Whole seed and chia meal | 0, 15%             | 46 days            | Productive performances, meat fatty acid profile                                     | Azcona et al., 2008              |
|                | Whole seed      | 0, 5, 10%            | 6 weeks             | Growth performance and body tissues fatty acid profile                               | Salazar-Vega et al., 2009        |
|                | Whole seed      | 0, 3, 6, 9%          | 6 weeks             | Meat fatty acid profile, long-chain omega-3 FA deposition                           | Komprda et al., 2013             |
| **Ruminants**  |                 |                      |                     |                                                                                      |                                  |
| Dairy cow      | Whole seed      | 0, 7.5%              | 94 days             | Total fat content, cholesterol content, FA composition of milk                       | Ayerza and Coates, 2006          |
| Dairy goat     | Discarded seed  | 100% maize concentrate replacement | 5 weeks           | Milk yield, milk gross properties, FA profile                                       | Martinez, 2013                   |
|                | Whole seed      | 0, 2.7, 5.5%         | 20 days             | Feed intake, digestibility, milk FA profile                                         | Schettino et al., 2017           |
| Lamb           | Whole seed      | 0, 10%               | From 16 to 27 kg live weight | Meat FA composition, sensory analysis                                              | Insausti et al., 2011            |
|                | Whole seed      | 0, 10%, 10% chia + 10% flax | Initial weight not specified, slaughtered at 27 kg live weight | Growth and carcass parameters                                                       | Mendizabal et al., 2011          |
|                | Whole seed      | 0, 10%               | 4 weeks             | Growth, carcass parameters, FA profile, regulation of genes lipogenesis             | Urrutia et al., 2015             |

Continued on next page.
in animal nutrition were published in the area of poultry science, which also show the largest variability in terms of seed products: materials range from whole seed - the most common type of supplementation - to chia flour and oil. Also in this area a few trials compared the effects of chia addition with other common sources of omega-3 such as linseed. So far toxicity or increased mortality have never been reported, even when chia seeds simply replaced a portion of control diet on dry matter basis. Ayerza and Coates (1999) showed that a 30% inclusion (without balancing protein/energy supply among diets) slightly reduced total egg yield (from 11.57 of control to 10.36 eggs day⁻¹ of chia diets, values averaged across dates) but greatly improved egg FA profile by reducing saturated fatty acids and increasing omega-3 content, no increased mortality was found. Palmitic fatty acid content of yolks was significantly reduced with the chia diet (P<0.05), after 4 weeks of supplementation the concentration was reduced by up to 35%. On average no significant effects on yield cholesterol were found but there was a tendency for a higher cholesterol content in the eggs produced by chia-fed animals; as commented by the authors this increase possibly reflects the different oil content of the two diets (6.67% vs 14.5% of control and chia diet respectively). ALA content was greatly increased (on average from 0.28 to 12.72% in the chia group), omega-3 to omega-6 ratio increased from 0.01 of control to 0.58 in the chia group. According to these authors with basis on scientific literature on linseed supplementation, chia seems more efficient than linseed in reducing saturated fatty acids. They conclude that chia supplementation could contribute to reverse the declining per capita consumption of eggs and egg products occurring in recent years thanks to improvements in egg nutritional profile without affecting organoleptic quality. A slight egg yield reduction was also reported by Salazar-Vega et al., (2009) when 7.5 and 15.0% chia inclusion were supplemented for 16 weeks to Babcock laying hens in comparison with a standard diet, in their case though egg weight increased with chia increase. ALA proportion increased significantly with increasing chia content (respectively 0% under control diet and 1.9 and 4.4% for the 7.5 and 15% inclusion), no significant differences between chia groups were detected. Chia did not modify linoleic acid or monounsaturated FA content, only at 15% inclusion level there was a slight reduction in C16:0. Consistent with the other studies reported here cholesterol content did not change. Another study showed that dietary chia effect on productive performances vary with the breed (Ayerza and Coates, 2000). Two breeds white and brown commercial strains (developed from White Leghorn and Red Sex Link breeds respectively) were fed 90 days with diets containing 0, 70, 140, 210 and 280 g/kg whole chia seed. In both strains chia did not affect hens weight and greatly improved lipid profile. Chia reduced yolk cholesterol content in white hens (this parameter was not measured on brown hens), from 1.01 of control diet to 0.93% at 28% inclusion. Docosahexanoic acid (DHA) was significantly higher (P<0.05) in chia groups. As reported by the authors white hens fed the 7, 14, 21, and 28% chia diets gave an average increase in omega-3 fatty acid content of 658, 1059, 1310 and 1659% as compared to brown hens whose eggs omega-3 content increased by 670, 1099, 1375 and 1682 in comparison with control diet. A significant (P<0.05) egg yield reduction though was observed in white hens at same dates (12% of total cases) in animals fed with the highest chia dose (21 and 28%), this lower production was coupled with a reduction of egg weight indicating that the 21% chia inclusion can be considered an upper limit for this breed. On the opposite chia increased egg weight for brown hens. With an average egg weight 65.75 g (no significant differences between chia diets). This way eggs produced by brown hens met the extra-large grade requirements (eggs weight >62 g) of Argentina egg market and no detrimental effects on organoleptic effect was observed. Interestingly in poultry science for the first time three studies compared chia dietary effect with linseed that is one of the most common plant-based omega-3 sources in livestock science (Ayerza and Coates, 2001; Antruejo et al., 2012; Coorey et al., 2015). Ayerza and Coates (2001) tested the effect of 5 combinations of chia and flaxseed (0-0, 7-3, 9-5, 11.5-2.5, 14-0% of chia and linseed respectively). Overall oilseed enrichment did not change egg yield and cholesterol content but improved FA profile. When the total inclusion rate of chia + linseed reaches 14%, palmitic acid content decreased and monounsaturated and polyunsaturated FA both increased (p<0.05). Oilseed inclusion decreased dramatically omega-6:omega-3 ratio, no significant differences were found among enriched diet. In general though the higher the chia content the lower the omega-6:omega-3 ratio that for the yolk ranged between 11.30 for the control diet to 1.76 for the 14% chia diet. The diet with the highest level of flaxseed (5%) reduced hens weight but yielded a significantly higher proportion of long-chain omega-3 DHA compared to the other chia diets and the control (p<0.05). The taste panel showed that the diet with the highest lin-

### Table 6. Continued from previous page.

| Animal          | Feed ingredient                  | Inclusion rate (w/w) | Experiment duration | Evaluated parameters                                                                 | Reference        |
|-----------------|----------------------------------|----------------------|---------------------|--------------------------------------------------------------------------------------|------------------|
| Fishes          |                                  |                      |                     | Nobody                                                                                 |                  |
| Nile tilapia    | Whole seed grinded               | 0, 5%                | 45 days             | Growth performances, lipid composition, FA profile                                   | Silva et al., 2014|
|                 | Seed oil                         | 0, 0.63%             | 60 days             | Lipid composition and antioxidant capacity evaluation                                 | Carbonera et al., 2016|
|                 | Chia oil alone or blended with avocado peel extract | 0, 1.9%, 1.9%+0.14% avocado extract | 45 days | Growth performances, meat FA profile, feed antioxidant capacity                      | Mostanber et al., 2016|
| Edible insects  |                                  |                      |                     | Nobody                                                                                 |                  |
| Cricket         | Whole seed grinded               | 0, 50%, 100%         | 10 days             | FA profile                                                                           | Kompreda et al., 2013|
| Giant mealworm beetle | Whole seed grinded | 0, 50%, 100%         | 10 days             | FA profile                                                                           | Kompreda et al., 2013|

FA, fatty acid
seed inclusion had a slightly lower performance. The fishy flavour associated with linseed dietary inclusion has been frequently reported (Caston et al., 1994) this work however shows that FA composition can be improved by mixing chia seeds with a low dose of linseed (<5%) (and thus reducing any side effects associated with linseed) this way organoleptic quality and productivity are not affected while nutraceutical quality is improved. Antrujo et al. (2011) compared the dietary effect of chia (oil and seed) and flaxseed (oil and seed) on egg quality. Chia oil and chia seed outperformed flaxseed yielding 54.5 and 63.5% more mg of ω-3 fatty acid per g of yolk during a test period of the 56 days, and 13.4 and 66.2% more for the 84 d test period, than flaxseed oil and flaxseed, respectively. Finally in the work of Coorey et al. (2015) chia flour was compared flaxseed and marine oils. A diet containing 30 and 40% of chia flour was more effective in increasing ALA content compared to flaxseed oil or marine oil, the best results in terms of quality were obtained at 30% (highest eggs ALA content) while at 40% inclusion eggs colour obtained the lowest score at panel test analysis. However LC-omega-3 eicosapentaenoic (EPA) and DHA were only found in eggs from hens fed with marine oil. Chia has also been tested on quails eggs quality (Komprda et al., 2013) at three inclusion rates (0, 5.5% and 7.5% w/w). One of the novelties in this study is that the dietary effect of chia was also quantified in terms short-chain to long-chain omega-3 biocconversion efficiency. In agreement with findings on hen eggs ALA content increased with increasing chia content, from 125 mg/100 g of control group to 284 and 395 mg/100 g for chia diets. Part of this ALA was converted into long-chain omega-3. LC-omega-3 EPA+DHA increased from 85 mg/100g of control to the 96 and 106-mg/100 g of the 5.5% and the 7.5% chia diets respectively. PUFA-n/PUFA-3 ratio in quails egg decreased linearly with increasing dietary ALA content (p<0.05). Several studies addressed the effects of chia on poultry meat quality and productive performances, in agreement with the results obtained on laying hens chia inclusion in a few cases slightly affected animals growth rate, no toxic effects were reported and all studies converge on the positive effects on meat FA profile. Ayerza et al. (2002) compared the effects on broiler meat quality of two chia-enriched diets (10% and 20% chia seed inclusion) to a control diet for 7 weeks. By the fourth week of supplementation feed conversion ratio was significantly higher in chia groups (1.96 vs 2.12 for control and chia at 20%), overall chia diet reduced body weight up to 6.2% (at 20% chia inclusion). According to the authors however this reduction is well below the 17.3% reduction reported by in literature for broiler meat. The study shows that even a 10% inclusion was sufficient to improve meat FA profile, in agreement with Ayerza et al. (2002) palmitic acid decreased and ALA increased significantly. Azcona et al. (2008) compared the effects of several diets (rapeseed, flaxseed, chia seed and chia meal) and found that two chia diets including ground seed or either chia meal (at 15% inclusion) increased meat ALA content. Chia seed gave the highest ALA increase (by 157% and 200% increases for dark and white meat respectively). All seeds except rapeseed increased tissues ALA, LC-omega 3 and total PUFA however the highest PUFA content was obtained supplying chia seeds. Flaxseed negatively affected productive performances by reducing body weights, weight gain and feed conversion ratios as compared to other feeds. Overall all of the omega-3 rich diets significantly reduced saturated fatty acids and omega-6:omega-3 ratio, but the study proves that these oilseeds are not biologically equivalent. Komprda et al. (2013) found that 6% of ground chia seed increased ALA content in broilers meat increased from 15 and 30 (mg/100 g fresh weight) of control (breast and thigh respectively) to 239 and 237 (mg/100 g fresh weight) measured in the two tissues compared to the chia group. Part of this ALA was converted into long-chain omega-3 in a proportion that was a function of the dietary ALA content. Namely increasing dietary ALA content resulted in a lower biocconversion efficiency: when a dietary ALA accounted for 50 mg/100g feed almost 6% was converted into DHA incorporated in edible tissues, while at 1340 mg/100 g this percentage decreased to 0.4%. Regarding docosapentaenoic acid (DPA) and EPA the authors showed that an increase of 1 mg dietary ALA for 100 g correspond to 0.36 mg of EPA and 0.17 mg of DPA deposited in broiler meat. They concluded that in order to enrich chicken meat with long-chain omega-3 the optimal chia inclusion level is 60 g kg⁻¹. Overall all studies published in poultry science show that dietary chia can greatly improve the FA profile of meat an eggs increasing their nutraceutical value and possibly their marketability. A slight reduction of productive performances at high chia doses was attributed to chia seed fibre content indicating that a 20% inclusion can be considered an upper limit at least for certain strains. The comparison with linseed diets showed that chia is a sound alternative or a complementary omega-3 source. Based on these data chia can be considered a safe ingredient capable of improving poultry products quality even when very small amounts (6% w/w) are added to the diet.

### Chia use in rabbit nutrition

The majority of research work on chia potential in animal nutrition has been developed in poultry science, encouraging results though have been obtained on rabbits for which chia has been tested in various forms, supplements include: pelleted seeds, chia oil and discarded seeds (Table 6). While the wide range of feed sources hamper the comparison between experiments, these studies open ground on the use of chia co-products (oil) and by-products (discarded seeds) in rabbit nutrition to improve both products quality and animal health status. Dietary use of whole chia seeds in rabbit nutrition were first studied by Meineri and Peiretti (2007) with the purpose of assessing chia effects on feed digestibility. Three isocaloric, isonitrogenous diets were compared containing 0, 10% and 15% (w/w) chia whole seeds respectively, all diets pelleted fresh. The control diet was a standard formula containing soybean oil and palm oil, chia totally replaced palm oil (4%) and partially replaced soybean oil (12% in control and 11% and 10% in low and high dose chia diets). The study demonstrated that up to 150 g/kg feed of chia seeds can be safely added to rabbit nutrition.
diet leading to an increased digestibility of dry matter, organic matter and gross energy compared control diet. While digestibility of crude protein, crude fibres and neutral detergent fibre did not differ from control, at the 15% chia dose acid detergent fibre digestibility did not differ from control but was significantly lower than the values measured for the 10% diet. Another study (Peiretti and Meineri, 2008) more extensively investigated the effect of these three chia-enriched diets on growth performances, carcass traits and meat nutritional quality. Up to 15% chia inclusion did not impair growth performances and did not alter carcass characteristics (i.e. carcass yield, commercial carcass weight). Rabbits final live weight, daily and total weight gain as well as total feed consumption and feed conversion efficiency did not differ from control diet. Chia most important effect however was exerted on meat FA profile that was greatly improved through a significant decrease (p<0.05) of saturated fatty acids and a dramatic dose-dependent increase of PUFA content. The high ALA content in chia seeds increased omega-3 PUFA content in both longissimus dorsi muscle (by 318 and 404% at 10% and 15% inclusion levels respectively, p<0.05) and in perineal fat (by 389 at 10% and 465% at 15%, p<0.05). A four-fold reduction of omega-6 to omega-3 ratio was also recorded (from 4.55 in control to as low as 1.03 at 15% for longissimus dorsi muscle and from 4.20 to less than 1.00 in perineal fat). Chia in comparison to a standard formula improved rabbit meat nutritional quality by halving the atherogenic Index in both meat (from 0.68 to 0.37) and adipose tissue (0.73 to 0.33). Thrombogenic Index was reduced to less than one-third (from 0.93 to 0.28 of control and 15% chia dose respectively), a similar trend was observed in perineal fat (from 0.97 to 0.22). Finally Meineri and co-authors (2010) studied the effects of these same chia inclusion rates (0, 10 and 15%) on meat quality, oxidative stability and sensory traits. Chia did not impaired meat gross properties (water, protein, lipid and ash content did not change), the pH measured 60′ after slaughtering was significantly higher in chia diets (pH<sub>max</sub>=6.72 > pH control =6.26; p<0.05) but the difference disappeared after 24 hours. However compared to control diet cooking losses increased in chia groups. One of the most important results of this study is related to the dietary effect of chia on meat oxidation stability. At 15% chia inclusion meat showed a significantly higher (p<0.05) susceptibility to lipid oxidation when refrigerated at 4°C (measurements taken at different dates over 2 weeks) and when frozen at –20°C (measurements taken after two months). The lower meat oxidative stability indicate that probably chia seeds antioxidant are not sufficient to protect meat PUFA from oxidation and that additional antioxidant inputs such as vitamin E could be considered (Castellini et al., 1998). The use of chia by-products has been tested for the first time in rabbit nutrition by Rodriguez-Abello et al. (2016) who studied the dietary effect of discarded seeds at inclusion rates of 10%, 20%, 30% and 40% w/w in comparison with a standard formula. Discarded seeds are the residues obtained after mechanical harvesting and pre-cleaning of seeds, mainly consist of light weight seeds and floral fragments, according to these authors they can constitute up to 10-15% of the total harvest. Clearly this low-cost by-product compared to commercial seeds showed a lower protein (12.3% vs 21.5%) and fat content (6.9% vs 28.8%), a higher fibre content and a lower digestibility. Despite the relatively low nutritional quality of discarded seeds the trial results were encouraging; no significant differences in the final body weight and body weight gain were observed between chia diets and control diet, nor among chia inclusion levels, on the other hand discarded seeds lowered the cost of feed ration (from 0.27 US$/kg of control to 0.23 US$/kg for chia diets). Chia proportions affected feed metabolism. Feed intake (p<0.05) increased linearly up to 20% inclusion (91 g/rabbit/day) and declined at 30% and at 40% inclusion level. Energy conversion ratio was significantly higher at 20% inclusion compared to the other diets and the control. These differences can be attributed to the increasing proportion of fibres (that range from 14% in control to the 20% of 40% chia dose) and fat (between 4.1% in control and 5.6% in 40% inclusion level, with the lowest value of 3.5% found in the 20% dose). Diet economic efficiency calculated as a function of weight gain and feed cost showed significant differences between treatments (p<0.05) being significantly higher at 30% and 40% inclusion rates than control and 10% and 20% inclusions. Overall the study demonstrated that diet economic efficiency can be increased by adding discarded chia seeds at inclusion rates between 30% and 40% but the effect on meat quality remain to be tested. Only one work analysed the use of chia oil (at 10% inclusion) as a functional feed for hypercholesterolemic rabbit (Sierra et al., 2015). The study showed how a hypercholesterolemic diet alters the vascular functions and how supplementing chia oil for 6 weeks was sufficient to attenuate triglycerides rise and increase plasma ALA contributing to partially restore the impaired vascular functionality.

### Chia use in pig nutrition

To the best of our knowledge only one work investigated the effect of chia supplementation to finishing pigs (Coates and Ayerza, 2009), in this work chia whole seeds were included in pigs diet at inclusion rates of 0%, 10% and 20% for 63 days As found for other species chia enrichment had no adverse effect on animal health and growth performance and positively affected meat FA profile. These positive effects however were large and significant on subcutaneous adipose tissue while no significant changes were detected on perineal fat deposits. Both 10% and 20% chia inclusion levels reduced meat fat palmitic acid content as well as total saturated FA (up to 23%) without significant differences between chia diets. Linoleic acid increased considerably in chia groups, a five-fold increase was observed without significant differences between diets. Meat fat ALA content at 20% chia inclusion increased as much as by 333%. Sensory analysis revealed consumers preference for chia enriched meat: aroma and flavour scored significantly higher for the 10% group, no adverse effects were associated to 20% inclusion however. The positive or not negative score attributed to chia enriched products, in line with data on other species, is one of the point of strength of chia seed use in animal nutrition since the off-flavours associated to other popular omega-3 rich oilseeds such as linsedge, falseflax or to fish-oils frequently reduce consumers acceptability.

### Chia use in ruminants nutrition

There is a great interest in dairy industry in improving the FA profile of milk and cheese by increasing PUFA context, as it is well recognised that saturated FA are commonly associated to an increased risk of cardiovascular system failure (Hu et al., 1999). Despite this interest and the relative wide diffusion of chia for rabbits and broilers diets, limited research has been conducted on dairy animals. To the best of our knowledge only one work tested the effects of chia inclusion in lactating cows (Ayerza and Coates, 2006) and very recently chia has been tested on lactating goats (Martinez, 2013; Scattini et al., 2017). In Holstein cows a 17% chia inclusion has been evaluated as a partial replacement of concentrate and oilseeds (replacement on dry matter basis) was tested as an alternative to soybean meal (10% in control diet) and as a partial replacement of maize and wheat flour (Ayerza and Coates, 2006). The resulting chia diet had lower protein content (14% vs...
18% of control) and twice as much as the fat content of the control diet. Despite these differences milk yield was not significantly lowered in chia group, and no significant differences were found in cholesterol content and total fat. Chia diet contained as much as 82 times the ALA of the control, however ALA content in milk only increased by 20%, possibly due to rumen bio-hydrogenation. Schettino et al. (2017) tested the effect of three inclusion levels of chia (0, 2.7% and 5.5%) in the diet of Saneen goats during the last third of the lactation period, animals belonging to the control group were fed with a iso-energetic, iso-nitrogenous control diet (160 g of crude protein/d and 11 MJ of metabolisable energy/d), based on barley hay and corn silage supplemented with concentrate (corn and soybean meal). Chia diets did not alter milk yield and gross properties compared to control diet, therefore in agreement with the data on dairy cows, no adverse effect on productivity was observed. The lower chia dose (2.7%) was not sufficient to produce sizable effects on animal performances or products quality while the 5.5% dose, compared to control diet, resulted in a higher feed intake (>nitrogen, >DM, >OM) and significantly improved milk FA profile. Total saturated fatty acids were reduced by 3.4%, mainly through the decrease of medium chain FA, while short chain FA did not change. The higher chia inclusion level corresponded to a higher milk MUFAs content (+19%, p<0.05), this was mainly related to the higher content of C18:0 and C18:1n-9 cis resulting from the rumen biohydrogenation of both ALA and linoleic acid, whose content in milk did not differ from control. With the highest chia dose PUFA content increased by as much as 20%, both inclusion levels were capable of increasing conjugated linoleic acid (CLA) isomers. Specifically total CLA content increased from 0.33 to 0.73% with chia diet. Overall the high dose of chia decreased the atherogenicity index by as much as 25%. Martinez et al. (2013) supplemented dairy goats with 700 g discarded chia seeds (52% purity, equivalent to 360 grams of chia seeds per goat per day) and compared this diet with a 400 g supplementation of corn concentrate, despite the perspective imbalance between diets (due to the iso-dry matter replacement) productivity was not affected. Consistent with other trials milk nutraceutical quality was greatly improved: atherogenicity index that decreased by 36%, short chain SFA did not change but C12:0, C14:0 and C16:0 decreased significantly (p<0.05) by 26%, 26% and 22%, respectively. Vaccenic acid increased by 133% together with CLA, 9cis;11trans isomer (+97%), ALA and linoleic acid increased by 45% and 61% respectively, these relatively modest increase are attributable to rumen bio-hydrogenation, as also suggested by the relatively high content of C18:0 (13.56% vs 9.94% in chia and control diet respectively). Chia dietary inclusion was also tested in lamb fattening (Table 6), in agreement with results obtained for other species chia dietary addition did not impair animal growth and improved meat FA profile. Mendizabal and co-authors (2011) tested the effects of oilseeds (chia and linseed) on lambs growth and carcass quality. A 10% chia seed inclusion and a 10% linseed supplementation were compared with a standard soybean-based commercial formula. Both chia and linseed did not alter Andorra lambs growth rate and carcass quality compared to control diet, sensory analysis revealed that animals fed with chia had a slight change in fat colour (yellow shade) while in linseed group fat colour was described as white and luminous. Interestingly however another study showed that at 10% levels of inclusion both chia and linseed greatly improved lamb meat fatty acid profile compared with a standard soybean-based control (Insamuti et al., 2011). No significant differences were found between chia and linseed-fed animals. Both chia and linseed significantly (p<0.05) increased meat ALA content (g/100 g total FA) rising it from the 0.52 of the control group to the 1.84 and 1.73 of linseed and chia-fed lambs respectively. Long-chain omega-3 EPA and DHA (g/100 g total FA) also increased slightly compared to control diet (0.21 EPA control diet <0.42 EPA linseed =0.36 EPA chia, p<0.05; and 0.46 DHA control diet <0.6 linseed =0.54 chia, p<0.05). DHA did not change. This omega-3 enrichment positively affected omega-6/omega-3 ratio that was nearly halved (4.56 of linseed=4.88 of chia, p<0.05) compared to the control (9.5). While chia and linseed inclusion were equally important in improving FA profile the sensory analysis revealed consumers preference for the meat produced with animals fed with chia diet (p<0.05) a result consistent with those obtained by Ayerza and Coates (2009) when chia was tested in finishing pig. Differences in Navarra lamb FA profile induced by flaxseed (raw and extruded) and raw chia seeds were investigated by Urrutia et al. (2015) who tested a partial concentrate replacement with either 10% (w/w) chia raw seed or a 10.5 % (w/w) linseed formula (containing 70% extruded linseed and 30% wheat bran). In agreement with other studies both chia and linseed partial replacement had no adverse effect on productive performances and improved meat FA profile, namely both chia and linseed supplementation increased ALA, eicosapentaenoic acid (EPA, C20:5n-3) in of the intramuscular and subcutaneous adipose tissue (p<0.001), the n-6/n-3 decreased from the 6.26 of control group to the 3.12 and 3.87 of linseed and chia respectively (p<0.001). Linseed increased DPA in both adipose tissues while chia increased DPA only at intramuscular level. These authors also found differences between linseed and chia on the expression of several genes involved in lipogenesis that could contribute to explain why chia and linseed omega-3 enrichment was tissue-specific. Chia seeds ALA content was higher than that of linseed (47.5% vs 33.7% respectively), nevertheless the concentration in meat was similar, suggesting that chia ALA possibly underwent through a higher rate of rumen bio-hydrogenation. Overall this first results on ruminant point to a relatively low Chia-ALA incorporation rate due to rumen bio-hydrogenation, therefore seed protecting technologies/antioxidant enrichment should be considered. Overall the results on ruminants indicate that Chia even at low doses (10%) can improve the quality of products without adverse effects on growth parameters.

**Chia feed in aquaculture**

Recently chia supplementation has been introduced in aquaculture. Dietary chia effects have been tested on freshwater fish Nile tilapia (*Oreochromis niloticus*) (Table 6). Silva et al. (2014) used for the first time chia in aquaculture. Specifically chia bran at 5% inclusion was tested as an alternative to soybean oil. After 45 days of chia supplementation, ALA incorporation increased by 876% compared to the 104% increase under control diet. Fillet EPA and DHA increased from 1.12 to 1.56 (mg 100 g<sup>-1</sup>) and from 19.55 to 26.55 (mg 100 g<sup>-1</sup>) respectively. Chia supply decreased saturated fatty acid (SFA), increased PUFA/SFA ratio (from 0.79 to 1 in chia diet) and a reduced omega-6/omega-3 ratio (from 12.07 to 3.77). In another study (Carbonera et al., 2016) chia oil has been evaluated as a lipid source, comparing a control diet in which the lipid fraction was provided by sunflower oil (1.90% inclusion) and chia-enriched diet in which sunflower was replaced by a blend of 0.63% chia oil, 0.63% tung oil, and 0.63% synthetic supplement of conjugated linoleic acid, the chia diet was also supplemented with 0.015% vitamin E. Clearly diet formulation (blend of different lipid sources supplemented with antioxidant) does not allow to discern the effect of chia alone on FA and antioxidant profile, except for ALA incorporation (since chia was the only source), as found for other species chia addition increased 2.9 times ALA content...
plants changes with growth stage from early vegetative stage to late vegetative stage to fall at 76 (g/kg DM) at 122 (g/kg DM) at late vegetative stage to fall at 76 (g/kg DM) at late vegetative stage. The stage before shooting is the best time for harvesting forage with a good nutritive value. The protein content in chia herbage at early vegetative stage (188 (g/kg DM basis) at early vegetative stage). The high nutritional quality of chia herbage at early vegetative stage suggests another potential use of chia in animal nutrition in the form of sprouts. Dal Bosco and co-authors (2015) showed that alfalfa and linsseed sprouts supplemented to rabbits improved meat fatty acid profile by increasing PUFA and decreasing omega-6:omega-3 ratio and the throbogenic index.

Chia use in edible insects nutrition

Dietary chia effects was tested on two species with an established use in human diet: cricket (Gryllus assimilis) and giant mealworm (Zophobas atratus) at the last nymphal stadium and at the last larval stadium respectively (Komprda et al., 2013). In both cases feeding on chia as an alternative to wheat bran increased dramatically ALA content. In cricket ALA increased from 12 to 3229 mg/100 g fresh weight at 50% inclusion and up to 3460 mg/100 g fresh weight for 100% diet, while in giant mealworm there was a relatively modest shift from 33 to 702 and 1520 mg/100 g fresh weight for 100% diet, while in giant mealworm there was a relatively modest shift from 33 to 702 and 1520 mg/100 g fresh weight at 50% and 100% inclusion respectively. Only cricket was able to synthesise long-chain omega-3 EPA. EPA increased in cricket up to 16 mg/100 g fresh weight similarly under both chia doses. The study proves that dietary consumption of chia-fed crickets can contribute to increase EPA intake in humans although a 100 g serving (corresponding to 20 mg of EPA) would only satisfy about 5% of recommended daily intake.

Chia whole plant as a forage source

As mentioned above there are no published work on the use of chia herbage in animal nutrition, however forage use of chia vegetative parts was suggested by Peiretti and Gai (2009), who determined a whole panel of chemical analyses relevant for animal nutrition. According to these authors chia plant biomass is a good source of polyunsaturated fatty acids (with ALA proportion varying between 649 and 565 g/kg total FA between early and late vegetative stage). The stage before shooting is the best time for harvesting forage with a good nutritive value. The protein content in fact varies between 188 (g/kg DM basis) at early vegetative stage and 122 (g/kg DM) at late vegetative stage to fall at 76 (g/kg DM) during shooting stage. Besides results on fatty acids and protein reported above, they found that forage quality of chia vegetative plants changes with growth stage from early vegetative stage to plant budding with an increase in fibrous fractions (acid and neutral detergent fibre and lignin) and gross energy and a decrease in fats and ashes. Another study (Peiretti, 2010) suggested that chia whole plant harvested at the budding stage has a good potential for large scale ensiling. Biomass values presented in section 2 show that whole plants can be grown successfully at all latitudes with high values even in Europe with short-day genotypes. Parameters related to ensiliability were determined by Peiretti (2010) who found a decrease in buffering capacity and an increase in water soluble carbohydrates from 86 to 213 g/kg of dry matter (DM), and significant variations in pH and soluble nitrogen with proceeding plant growth stage. In a lab-scale facility the author ensiled plants harvested at budding with four different methods (cut silage and wilted silages at three levels of wilting). Results indicate that some alcohols and volatile fatty acids (but no lactic acid) are produced during fermentation and only isobutyric acid decreased with increasing wilting level. He concluded that chia vegetative parts could be considered for ensiling if collected at the budding stage and wilted to more than 285 g/kg of dry matter. Bilalis et al. (2016) conducted an agronomic trial and found that fertilising with manure affects crude protein and both acid and neutral-detergent fibre. The high nutritional quality of chia herbage at early vegetative stages suggests another potential use of chia in animal nutrition in the form of sprouts. Dal Bosco and co-authors (2015) showed that alfalfa and linsseed sprouts supplemented to rabbits improved meat fatty acid profile by increasing PUFA and decreasing omega-6:omega-3 ratio and the throbogenic index.

Further prospects

Up to now research on chia in the livestock sector only addressed the dietary effects on products quality, but another potential use might be related to antimicrobial properties of chia extracts. Using ethanol extracts of seeds and green parts of chia Patil et al. (2014) showed the effect on paralysys and death of earthworms as a model for intestinal parasitic worms. Essential oils from chia leaves include β-caryophyllene, globulol, γ-murolene, β-pinene, α-humulene, germacrene-B, and widdrol (Ahmed et al., 1994; Elshafie et al., 2018). Ahmed et al. (1994) suggests that chia leaf oils could be used as insect repellent. Elshafie et al. (2018) tested essential oils on several fungi and bacteria; antifungal effect was important especially against Aspergillus fumigatus, Penicillium expansum, Monilia laxa, and M. fructigena; the antibacterial action of chia essential oils was strong against a number of Gram + bacteria. The antimicrobial activity of extracted essential oils shown by Elshafie et al. (2018) may be useful against bacterial and fungal contamination of forages. These effects coupled with a possible insect repellent capacity suggest that chia extracts may play a role in the prevention and treatment of parasitic burden.

Conclusions

The current body of knowledge on use in animal nutrition indicates that chia seeds can be considered an omega-3 boosting feed ingredient. Sizeable improvement of animal products fatty acid profile can be obtained even at low doses (6% w/w). If raw seeds are used, high inclusion rates (>20%) can slightly reduce productive performances, an effect possibly linked mucilage contained in the seeds which can act as a physical barrier hindering nutrients...
absorption at least in some species/breeds. The use of by-products such as discarded seeds can be a successful way to improve products omega-3 content at a very low cost. Studies on ruminants indicate that chia lipids undergo a substantial rumen bio-hydrogenation, and also a low oxidative stability of meat produced by rabbit fed with chia was reported, indicating that seed antioxidants might not be sufficient to protect PUFAs and that the addition of antioxidants supplements could help increase omega-3 incorporation rate. One of the points of strength of dietary chia is the total lack of anti-nutritional compounds coupled with the absence of undesirable off-flavours in the final products. Chia in livestock is essentially used as an omega-3 boosting feed. ALA proportion in seeds is relatively high (>60%) with genotype and environmental factors playing a major effect on the fatty acid composition rather than the total oil content. Seed yield of commercial short-day flowering varieties can be as high as 3 T ha⁻¹ in the countries of origin but is severely reduced at northern latitudes where the reduction of day length is accompanied by a decrease of air temperature that can compromise seed ripening process. Data on seed yield in Europe indicate that seed production would only be viable if genotypes with reduced photoperiod sensitivity will be released. Whole plant biomass and leaf biomass, on the opposite, can be remarkably high at European latitudes even with short-day flowering commercial varieties. Chia vegetative tissues display an ALA content between 649 and 565 g/kg total FA between early and late vegetative stage and are also a source of protein and secondary metabolites. The forage use of chia remains to be tested but data on forage quality and ensilability are encouraging. If whole-plant use in livestock sector will be validated, novel cropping opportunities will emerge for this crop in European crop-livestock systems even using commercially available short-day flowering varieties.

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