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Sustainable Biorefinery of Alfalfa (*Medicago sativa* L.): A Review

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ALFALFA is considered as "*the Queen of the Forages*" due to its high protein content and nutritional value. The production of alfalfa under different stressful environments is a great challenge, representing a serious threat to global food security. To improve the production of alfalfa under stressful conditions, there is a crucial need to understand the response of alfalfa plants to stresses, the mechanisms of tolerance and the management options. Alfalfa is a promising biorefinery crop from which leaf protein concentrates as well as bio-organic fertilizers can be produced. The potential of leaf-derived protein concentrate for human and animal nutrition is huge, yet unexploited. The bio-organic fertilizers derived from alfalfa plants represent a sustainable way to supply the plant with some nutrients. This review article highlights the sustainable use of alfalfa in biorefinery and bio-organic fertilizer production. Moreover, scattered knowledge about its use for production is collected supporting its suitability for improving soil fertility. Last, we discuss the suitability of alfalfa to produce stable yields in the light of changing climate and stressful conditions like salinity.

Keywords: Bio-organic fertilizers, Fiber, Leaf protein concentrate, Queen of forages.

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important legume forage crops in the world and is primarily used as silage, hay and pasture to feed livestock (Hawkins & Yu, 2018; Patra & Paul, 2019). This plant also called "lucerne" in Europe and other countries and its sprouts can be used as a staple crop for animals and humans due to its impressive nutritional content, including vitamins (i.e., B, C, D and E), high protein content and other important minerals (Mattioli et al., 2019; Michalczyk et al., 2019). Alfalfa was originally cultivated in south-central Asia (modern Iran) and it is well known as "*the Queen of Forages*" because of its high biomass yield, good quality of its forage and its palatability for ruminants (Lei et al., 2017). Alfalfa can grow in a wide range of soils and under several growing conditions, including nutrient poor soils (Lei et al., 2017). More than 40 million hectares are cultivated worldwide (Luo et al., 2019). Alfalfa yields are higher in light-textured soil conditions (e.g., sandy loam, silty loam and clay loam) than in heavy textured soils (Kavut & Avcioglu, 2015; Mbarki et al., 2018). Alfalfa could be considered as one of the most important crops for sustainable agriculture due to its promotion of soil fertility, ability to feed livestock in mixed production systems, N-fixation rate and ability to reduce greenhouse gas emissions (Luo et al., 2018; Kulkarni et al., 2018). This plant can grow under arid and semi-

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arid conditions (200mm annual precipitation) due to its relatively deep root system and enhancement of antioxidative protection and declining lipid peroxidation as a crop that is tolerant to water deficit (Lei et al., 2017; Zhang et al*.,* 2019). Based on its use in the production of organic acids and ethanol, alfalfa has a great potential as a biofuel feedstock (Luo et al., 2019).

A green biorefinery can be defined as an innovative zero-waste approach, in which green biomass can be totally converted into bio-based products (Bastidas-OyanedelJens & Schmidt, 2019; Srivastava et al*.,* 2019). The green biomass of alfalfa plants could be converted into solid and liquid fractions through the mechanical fractionation producing leaf protein concentrate (Hojilla-Evangelista et al*.,* 2017; Jia et al*.,* 2017; Hadidi et al., 2019), fiber (Palmonari et al., 2014; Fustini et al., 2017), green and brown juice (Dietz et al*.,* 2016). Recently, a considerable literature has grown up around the theme of the sustainable use of fodder legumes for green biorefineries, in particular alfalfa (Papendiek, 2017; Lübeck & Lübeck, 2019; Sarnataro et al., 2019). Fodder legume crops play a vital role in the development and the sustainability of agricultural production systems due to their direct and indirect potential to produce several compounds including food, feed and biofuels (Papendiek et al*.,* 2016; Papendiek, 2017; Lübeck & Lübeck, 2019).

This study systematically reviews the data for alfalfa production with the goal to provide a comprehensive overview on biochemical and anatomical aspects of the alfalfa plant. Drawing upon stressful environment research into alfalfa, this study attempts to present the several benefits of this crop under stress and biorefinery processes.

Botanical Aspects, Economic Uses and Production of Alfalfa

Alfalfa is one of the oldest forage crops in the world, with the first cultivation occurring in ancient Iran more than 2000 years ago. Alfalfa moved from Iran to Greece about 500 B. C (Patra & Paul, 2019). The name "*alfalfa*" originated from the Arabic word "*al-fiṣfiṣa*" which was modified into the Spanish word "*alfalfa*". The alfalfa plant consists of a root system with a long taproot, leaves, stems, flowers and seeds (Fig. 1). It is a perennial flowering plant classified as a leguminous dicot crop. The root system of alfalfa plants is very deep and strong with several lateral roots, which help the plant penetrate the soil to deeper layers making it tolerant to drought (Fig. 1, Photo 3). The leaves of alfalfa are trifoliate. The alfalfa middle leaflet has a short petiole, which is the main difference between alfalfa and berseem, along with sharply toothed leaflets on the upper 1/3 of the leaf margin (Fig. 1, Photo 4). Alfalfa flowers may be yellow, blue, white or purple in color. The flowers are collected in a group or cluster as an inflorescence in a raceme type (Fig. 1, Photo 5). Alfalfa is typically cross-pollinated by insects, in particular bees. Alfalfa seed has the shape of a kidney, is yellowish brown in color and very light-weight (Patra & Paul, 2019).

The economic importance of alfalfa

The most important producers of alfalfa include the USA, Canada, Australia, Argentina, India, Italy, New Zealand, France and Russia (Wang et al., 2018). Alfalfa is a C_3 plant, a perennial legume, and may be continuously cultivated for 3–4 years as a green fodder crop. This plant also can grow under different climatic conditions ranging from arid to tropical regions as well as from below sea level to elevations of 2500 m altitude. Alfalfa is mainly cultivated in the winter season ranging from the end of September to early December, with the optimum cultivation date in the middle of October like in Egypt. The seeds of alfalfa, like other leguminous crops, need to be inoculated with the suitable *Rhizobium* strain (*R. meliloti*). To produce a high yield, alfalfa plants consumed a huge quantity of water during the long growing season. The irrigation interval of alfalfa ranges between 7 and 10 days and may be extended to 25–30 days (Patra & Paul, 2019).

The production of alfalfa produces very high green fodder yields (depending on variety, 80–100Mg ha^{-1}) for long times (3–4 years) and seed yield ranges from 0.3 to 2.0Mg seed ha^{-1} (Patra & Paul, 2019). Alfalfa crop has many benefits including its use in grazing, producing hay and silage, increasing soil fertility as green manure, and protecting soil as a cover crop. However, alfalfa has several economic uses that are detailed as follows:

(1) It is a regular source for green fodder, hay, and silage for livestock during the dry season as compared with other forages (Hawkins & Yu, 2018; Wang et al., 2018).

(2) The decomposition of alfalfa biomass in soils increases the soil fertility status due to the fixation of atmospheric nitrogen, increased humus content and nutrients, and improved soil biochemical properties (Jonker & Yu, 2016; Ju et al., 2019).

(3) Alfalfa has the ability to reduce nitrate leaching in soils due to its huge uptake of water that contains nitrates during plant growth (Yang et al., 2016). It can also reduce the loss of carbon and promote carbon sequestration (Hassen et al., 2017).

(4) Alfalfa has a remarkable deep root system (up to 3m depth) that supports plant growth under unfavorable environments due to the ability to uptake water and nutrients from a considerable depth (Patra & Paul, 2019).

(5) It is possible to harvest from 7 to 8 cuttings in a season of perennial alfalfa growth, where the first cut takes 55–60 days from sowing and the following cuts take place when the cultivated plants achieve a height of 60cm from the soil surface. Therefore, alfalfa is an excellent cutting fodder plant compared to other forage crops (Patra & Paul, 2019).

(6) Alfalfa has a high nutritional value, represented in the high minerals and vitamins content. Depending on the time of cutting alfalfa has low fiber and high-energy content (Patra & Paul 2019; Sarnataro et al., 2019).

(7) Alfalfa could be used for green biorefinery producing feed, fuel and fertilizer (Santamaría-Fernández et al. 2018; Sarnataro et al., 2019).

(8) Alfalfa pellets are a highly proteinaceous feed that contains high protein concentration (up to 20%), carotene, and energy for feeding livestock (Jonker et al., 2017; Patra & Paul, 2019).

(9) The silage of alfalfa is one of the its most important products when the green fodder is at less than 65% water content in the presence of anaerobic bacteria. Silage performance can be enhanced by improving the quality of fermentation by adding organic acids like malic, citric, lactic, acetic, and propionic acids to preserve the silage (Ke et al., 2017; Zhang et al., 2017; Patra & Paul, 2019).

(10) The greatest leaf yield is achieved when alfalfa is harvested at the budding stage, but at the early flowering stage both leaf and stem yields are nearly the same. The highest yield and nutrients content of alfalfa forage is achieved at the early flowering stage. At advanced maturity stages, there is a decrease in crude protein in the harvested forage and an increase in fiber content (Fan et al., 2018). The biomass yield of alfalfa could be improved by using biotechnological tools or genetic engineering approaches under stressful conditions (Lei et al., 2017; Gou et al., 2018; Lei et al., 2018; Singer et al., 2018). The handling of alfalfa fractionations could also be improved, such as reducing lignin content in the forage legume (Barros et al., 2019).

(11) The main use of alfalfa is to feed dairy cattle, characterized by high dairy production, due to the high protein content and digestible fibers. Alfalfa is also used to feed beef cattle, sheep, horses and goats (Broderick, 2018). Alfalfa hay is commonly used as a source of protein and fiber to feed meat rabbits (Dal Bosco et al., 2015; Khan et al., 2016). Alfalfa leaf concentrate and dehydrated alfalfa could be used for the pigmentation of eggs and poultry meat due to the carotenoids content. The main use of alfalfa for human nutrition is the sprouts, which can be used in sandwiches and salads (Keshri et al., 2019).

(12) The cultivation of alfalfa plants may improve soil infiltration rates, reducing runoff and thus the loss of highly productive soils that support production of cultivated crops. Increasing the alfalfa growing period is seen as an effective soil-erosion-control practice that can help reduce soil erosion rates by 20–30% (Hassen et al., 2017).

(13) Although there are 60 different legume crops, alfalfa is considered as an important source for forage and feeding animals (Kulkarni et al., 2018). Improving the forage digestibility of alfalfa may reduce methane emissions by 15–30% per animal product unit (Hassen et al., 2017).

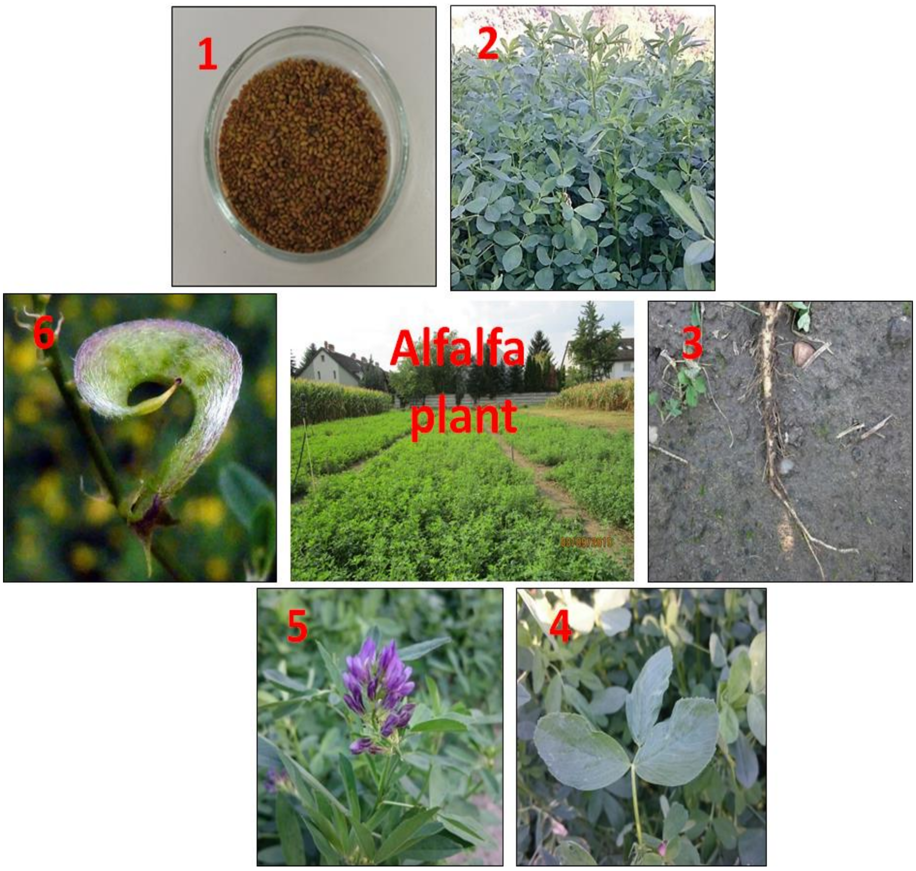

Fig. 1. General overview of the alfalfa plant starting with the seeds (Photo no. 1), the overall look of alfalfa in the field (Photo 2), the root system of the alfalfa plant (Photo 3), the distinguished leaf with the trifoliate leaves shape (Photo 4), the flower in inflorescence position and a raceme type (Photo 5), and finally the fruits of alfalfa as a legume (Photo 6)

Constraints of production and handling of alfalfa

On the other hand, there are also many constraints in the production and handling of alfalfa compared with other forage crops (Table 1). These constraints can be summarized as follows:

(1) Although its water requirement is quite high at about 500-1168mm (20-46 in) per season (Yang et al., 2019a), alfalfa is not tolerant to waterlogging (Zeng et al., 2019).

(2) Alfalfa is characterized as an out-crossing autotetraploid crop (2n= 4×= 32) with a genome size of 800–1000Mb and a basic number of 8 chromosomes, so challenges can be expected in efforts to improve its agronomic traits using breeding approaches (Hawkins & Yu, 2018).

(3) Alfalfa breeding could be used to improve agronomic and quality traits, yield, and its resistance to stress conditions as well as to enhance the digestibility of stems (Gruber et al., 2017). The breeding program could include conventional breeding techniques (e.g., selective and cross breeding), or applications of transgenic technology (Wang et al., 2016). The main problems for the breeding of alfalfa in general may include the slow progress rate, low success rate, the lack in breeding materials, low adoption rate of new cultivars, and lack of advanced breeding techniques (Wang et al., 2016; Shi et al., 2017). Increasing vegetative shoot branches is a desired outcome in alfalfa breeding as well as delaying the flowering time, as this approach would increase forage yield (Gao et al., 2018).

(4) It is expected that the increase in temperatures and drought conditions owing to climate change may occur more frequently in the future creating challenges for alfalfa production (Patra & Paul, 2019).

(5) Alfalfa genomics could be a considerable

challenge where they are highly heterozygous, which precludes any development in inbred lines and severe inbreeding depression (Hawkins & Yu, 2018).

(6) Alfalfa is generally self-incompatible, allogamous, and several insects can pollinate its flowers (Monteros et al., 2014). A little progress has been achieved in the incorporation of desired transgenic traits into commercial alfalfa although the ecological and economic importance of the allogamous and autotetraploid alfalfa crop (Jozefkowicz et al., 2018).

(7) The relatively high cost of alfalfa cultivation is one of the most frequently stated problems with its production. Cultivation requires relatively high energy consumption during agricultural operations such as sowing, harvest and post-harvest (Ghaderpour et al., 2018).

(8) Alfalfa proteins undergo rapid degradation, creating a risk of bloated rumens in ruminants. This problem also may cause a huge economic loss for farmers (Lei et al., 2017).

(9) The high lignin content in alfalfa also may link with the unbalanced degradation of nitrogen / carbohydrate ratio in the rumen of ruminants (Lei et al*.,* 2017). Therefore, there is an urgent need to develop and reduce lignin content in alfalfa (Gallego-Giraldo et al., 2016; Getachew et al., 2018; Barros et al*.,* 2019).

(10) Improving alfalfa productivity under biotic and abiotic stresses was and still is one of the most important strategies. Biotechnological techniques have been used to increase the resistance of alfalfa to disease and pests. The most common alfalfa root diseases include fusarium wilt (*Fusarium oxysporum*; Berg et al., 2017), verticillium wilt (*Verticillium alfalfae*; Xu et al., 2019), bacterial wilt (*Clavibacter michiganensis*; Singer et al., 2018) and phytophthora root rot (*Phytophthora medicaginis*; Samac et al., 2015). Studies that have investigated the diseases and pests of alfalfa include Nemchinov et al*.* (2017), Sisterson et al. (2018), Samarfard et al*.* (2018), Gaafar et al. (2019) and Li et al*.* (2019).

(11) Fetch alfalfa sprouts are prone to microbial attack by human bacterial pathogens (e.g., *Salmonella* spp., *Vibrio cholerae* and *Escherichia coli*). Suitable safety management levels are need to be established in support of human health (Kim et al., 2018; Cui et al., 2018; Mattioli et al., 2019). Sprouts could be preserved by soaking in resveratrol solutions and thymol (Lai et al., 2017), heat- and freeze-drying (Mattioli et al., 2019), hot water treatment (Michalczyk et al*.,* 2019), plasma-activated water (Xiang et al*.,* 2019) and gamma irradiation (Kang et al*.,* 2019).

Biochemical Characterization

The chemical composition of alfalfa, as with all plants, depends on the growth media in which the plant is established in addition to its structure. The plant's chemical composition is mainly related to quantitative nutritional components, which the plants may deliver to consumers (i.e., livestock or humans). This chemical composition also includes proteins, fats, fiber, carbohydrates and other micronutrients content of the plant tissues (Lee, 2018). Alfalfa forage is considered one of the most nutritious and palatable green fodders. This forage contains about 16–25% crude protein, which has 72% digestibility, along with about 20–30% fiber content and several vitamins (e.g., vitamins A, B, D, K and E) and minerals, such as calcium, potassium, iron, and magnesium (Patra & Paul, 2019). The chemical composition of cultivated or harvested alfalfa is a very important factor in alfalfa handling and its use as silage, forage or hay. Furthermore, the metabolic processes of alfalfa forage during fermentation have a great potential to change the nutritional composition of the standing crop (Santos & Kung, 2016).

The factors found to influence alfalfa chemical composition have been explored in several studies (e.g., Gaweł, 2012; Vintu et al., 2012; Santos & Kung, 2016; Hojilla-Evangelista et al., 2017; Rajabi et al., 2017; Ogunade et al., 2018; Guo et al., 2019; Hartinger et al., 2019). These studies included different factors affecting the chemical composition of alfalfa forage or silage as follows:

(1) Evaluation of the impacts of wilting intensity, dry matter level and the addition of sucrose on nitrogen compounds and fermentation products in alfalfa silage. This study confirmed that water-soluble carbohydrates may have a crucial role in both the alfalfa fermentation silage and the preservation of proteins (Hartinger et al., 2019).

TABLE 1. A comparison of maize fodder crop with two leguminous fodder crops (alfalfa and berseem or Egyptian clover)

Item of comparison	Maize	Alfalfa	Egyptian clover
Scientific name	*Zea mays* L.	*Medicago sativa* L.	*Trifolium alexandrinum* L.
Common name	Maize and corn	Alfalfa and lucerne	Berseem clover, Egyptian clover
Plant origin	Mexico and Central America	Probably originated in Iran	Probably originated in Syria
Crop life cycle	Annual crop (6–7 months)	Perennial crop (3 – 4 years)	Annual crop (6–7 months)
Main crop kind	Cereal grain C4 crop	Leguminous C3 fodder or forage crop	Leguminous C3 fodder / forage crop
Family	Poaceae	Fabaceae	Fabaceae
Root system	Adventitious fibrous type	Deep root system (up to 3m)	Shallow taproot
Main uses of crop	Biogas, green fodder, feed for livestock	Green fodder, hay and silage	Green fodder and silage
Green fodder yield	30–50Mg ha^{-1}	80–100Mg ha^{-1}	1.0–1.2Mt ha^{-1}
Seeds or grain yield (Mg ha^{-1})	8–11 Mg ha^{-1}	300 – 2000kg ha^{-1}	340kg ha^{-1}
Inoculated bacteria strain	No inoculation	*Rhizobium meliloti*	*Rhizobium trifolii*
Number of cutting per season	Maize silage or stover	7-8 cuts during season	4-6 cuts during winter
Chemical composition of green forage (based on DM)	10–12% crude protein, 25–30 crude fiber	16–25% crude protein 20–30% crude fiber	12.8% crude protein 26.7% crude fiber
Drought stress	Sensitive to excess moisture stress or water logging	High drought resistant	Less drought resistant
Soil alkalinity and water-logging stress	Sensitive to waterlogging and soil alkalinity	Sensitive to waterlogging and soil alkalinity	High tolerant to moisture and soil alkalinity
Soil salinity stress	Moderately sensitive	Moderately sensitive	Sensitive
(EC @ 50% yield)	(EC= 8.6dS m^{-1})	(EC= 8.8dS m^{-1})	(EC= 5.7dS m^{-1})
Main producing countries	USA, China, Brazil, Mexico. Argentina and India	USA, Canada, Australia, Argentina, India, Italy, France	Egypt, Syria, Pakistan, and India
Preferred soil	Loamy sand to clay loam	Light textured soils	Heavy-textured soils
The best time for sowing	June in kharif season	Middle of October	Start from November to May
The optimum seed rate	50–75kg ha^{-1} as a fodder crop	20–25kg ha^{-1} (in broadcast)	25–30kg ha^{-1}
Fertilizer requirements	80kg N ha^{-1}, 40kg P_2O_5 ha^{-1} and 20kg ha^{-1} $ZnSO_4$	20kg N ha^{-1}, 60–75kg P_2O_5 ha^{-1} and 40kg K_2O ha^{-1}	10kg N ha^{-1}, 80–90kg P_2O_5 ha^{-1}, 30–40kg K_2O ha^{-1}
Irrigation interval	7–10 days	7–10 days	15–20-day
Water requirements	5000m^3 water/ha	7500m^3 water/ha per season	4500m^3 water/ha

Sources: Barik & Hedayetullah (2019), FAO (2019), Patra & Paul (2019), Sah et al. (2019).

(2) The impact of harvesting time on the quality of silage fermentation and its digestibility. Alfalfa plants were harvested at initial flowering, the budding and full flowering stage then preserved as silage or ensiled for 45 days. With advancing maturity of alfalfa silage, the crude protein contents were decreased, whereas the content of dry matter, acid detergent fiber and neutral detergent fiber were increased (Guo et al., 2019).

(3) The application of citric or malic acids on the

quality of the fermentation process and the chemical composition of alfalfa silage. High quality alfalfa silage was produced using lactic acid bacteria in order to enhance the rapid decline of silage pH, improving the quality of fermentation-ensiled forage. Malic and citric acids may accelerate the growth of lactic acid bacteria during the fermentation of alfalfa silage (Ke et al., 2017, 2018a, b; Li et al., 2018).

(4) The role of moringa leaves in enhancing the fermentation quality and nutrition of alfalfa silage through mixed ensiling of alfalfa and with the leaves of *Moringa oleifera*. It was thought that, the moringa leaves could stimulate some microbial communities (e.g., *Enterobacter* and *Lactobacillus*) and inhibit some undesirable bacteria such as *Escherichia*, *Bacillus*, and *Staphylococcus* during the fermentation process (Wang et al., 2019).

(5) The effect of sterilization during the ensiling of alfalfa silage. Some inoculants like *Lactobacillus plantarum* were used during the fermentation of alfalfa silage to enhance the lactic acid fermentation and improving the silage quality (Yang et al., 2019b).

(6) There are many additives that can be used to improve the silage quality of alfalfa such as organic acids (e.g., formic, acetic, malic and citric acid; Yuan et al., 2018; Ke et al., 2018a, b) and salts such as calcium propionate (Dong et al., 2017), sodium diacetate (Yuan et al., 2017) and potassium diformate (Yuan et al., 2018), as well as jujube powder (Tian et al., 2017) and cumin essential oil (Turan & Önenç, 2018). These additives may enhance the transformation of nitrogen during the fermentation of alfalfa silage.

Results for the chemical composition of alfalfa silage before ensiling under different treatments are given in Table 2. A global comparison was carried out for 136 different forage plants looking at chemical composition and nutritive values under different environments (Lee, 2018). It was reported that not only are there distinguished variations among these forage plants in terms of the protein, lignin, fiber, and minerals content but also in the digestion rate by herbivores. This study also demonstrated that more than 90% of the plant's biomass may be digestible and the mean content of protein (2–36), lignin (1–21), fiber (23–90, as NDF or neutral detergent fiber) and minerals or ash (2–22) was recorded as a percent.

The Promising Biorefinery of Alfalfa Crop

Natural renewable resources and their sustainability have long been a question of great interest in a wide range of fields. Due to the scarcity of these resources, there is an urgent need to use them in the most sustainable and efficient way possible. Hence, the biorefinery process could allow both sustainability and efficiency of waste reduction under the exploitation of bioresources (Papendiek, 2017). The production of alfalfa forage has several beneficial and positive environmental impacts under highly efficient use in the bio-industrial feedstock industry as well as fodder in the framework of the green biorefinery approach (Papendiek, 2017). The biorefinery process was defined by Hassan et al. (2019) as "the sustainable processing of biomass into a spectrum of marketable products (food, feed, chemicals, and materials) and energy (fuels, power and/or heat)".

The great debate, generated by the first generation of biorefineries, was represented in the use of edible food crops to produce bioenergy. Recently, a considerable literature has grown up around the second generation of biorefineries to avoid using edible food crops in producing bioenergy (e.g., Hassan et al., 2019). The second generation of lignocellulosic biorefineries (i.e., lignocellulosic wastes and non-food crops) seeks to use the lignocellulosic biomass for bioenergy generation (De Bhowmick et al., 2018; Branco et al., 2019). The non-food crops used under this model include alfalfa, willow, poplar, and some grasses like reed canary, which are characterized by high productivity and their suitability to be produced in poor soils and marginal lands (Longato et al., 2019). The second generation also includes different agricultural residues such as rice husk and its bran, sugarcane bagasse, wheat straw, forest thinning, maize stover, wheat bran, and agricultural residues or wastes, e.g. apple pomace, orange and potato peels, groundnut and soybean wastes (Contreras et al., 2019; Hassan et al., 2019). However, it is important to realize these wastes are not "free" items, returning a certain amount of crop residues to the soils that produced those crops is critical to maintain soil quality; therefore, use of these residues for bioenergy must be done judiciously (Brevik et al., 2019).

TABLE 2. Some different values for the chemical composition of alfalfa fresh forage before ensiling according to different sources

Contents of alfalfa silage	Tao et al. (2017)	Ke et al. (2018a)	Yang et al. (2019b)	Wang et al. (2019)
Dry Matter, DM (g kg^{-1} fresh weight)	200	432	248	263
pH	6.51	6.21	6.43	5.12
Crude protein (g kg^{-1}, DM)	230	200	277	183
Neutral detergent fiber (g kg^{-1}, DM)	400	384	440	386
Acid detergent fiber (g kg^{-1}, DM)	312	287	207	278
Water soluble carbohydrates (g kg^{-1}, DM)	53.2	60.7	98.5	434

Leaf protein concentrate

Protein is a major component of interest within the field of human nutrition and health. It is essential to synthesize muscles, in cell signaling, immune responses and for repairing of damaged cells (Lonnie et al., 2018; Wen et al., 2019). The different sources of dietary protein should be sustainable including both plant-based and animal-based protein sources (Richter et al., 2015). Based on the Recommended Dietary Allowances, the daily average requirement for protein is 0.8 g protein per kg of body weight (Gardner et al., 2019). Pulses or leguminous seeds can provide about 33% of human dietary proteins as well as fiber, minerals, carbohydrates, several phytochemicals and vitamins (Bessada et al., 2019). Plant-derived proteins could be defined as energy concentrates or nutritional supplements, which have a wide range of functions in the pharmaceutical and food industries. Therefore, there is an urgent need for advances in renewable plant-derived protein sources (Wen et al., 2019).

Recently, a considerable literature has grown up around plant-based nutrition, in particular protein due to the differences between plant–based proteins and animal-based proteins (e.g., Patel et al. 2017; Morin et al., 2019). These differences include:

(1) The high intake of animal-based proteins causes many health problems like cardiovascular disease, diabetes and obesity.

(2) The water, land and energy requirements for the production and transportation of animal proteins are higher than plant-proteins.

(3) Plant-based proteins are more sustainable alternative due to lower emissions of greenhouse gases (Lonnie et al., 2018; Morin et al., 2019).

Dried alfalfa leaves contain 260 – 300g kg^{-1} crude protein on a dry basis (DB), while the stems have a lower content (100–120g kg^{-1} DB) (Hojilla-Evangelista et al., 2017). Proteins are primarily found in the mesophyll cells of leaves (Jonker & Yu, 2016). There are two fractions of soluble proteins in alfalfa leaves. The first group represents about 30 – 50% of soluble proteins in the form of ribulose-1,5- bisphosphate carboxylase/ oxygenase (Rubisco), while the second fraction of soluble proteins includes the plant hormones and enzymes as well as other protein components in small amounts. Apart from soluble proteins, the vacuole cells of alfalfa plants have non-protein-N or inorganic-N fractions (i.e., NO_3^- and NH_4^+), whereas the soluble protein (mainly Rubisco) is found in the plant's chloroplasts (Jonker & Yu, 2016). Therefore, the proteins in alfalfa may be considered an important source for animal nutrition (in the form of green forage and silage) and humans as sprouts and others (Mohammad et al., 2019). Alfalfa proteins could be introduced into human nutrition as products like sprouts, cookies, and other baked goods. Alfalfa protein concentrates could be also used as dietary supplements to improve human quality of life and health (Gaweł et al., 2017).

Alfalfa is one of the highest protein content forage crops, with leaf protein concentrations that range from 500 to 890 g kg^{-1} DB (Hojilla-Evangelista et al., 2017). Thus, there is great interest in extracting and producing protein from alfalfa leaves because it could be an inexpensive alternative source of food protein (Lamsal et al., 2007). Based on the biorefinery of alfalfa plants, concentrated protein could be extracted from dried alfalfa leaves using traditional techniques such as alkali solubilization for 2hrs at 50°C and acid precipitation (Hojilla-Evangelista et al., 2017). The leaf protein concentrate from alfalfa is characterized by its desirable emulsification, foaming, and gelling properties (Hojilla-Evangelista et al., 2017). On the other hand, there are some problems in handling alfalfa leaf protein concentrate in human foods. These constraints include the brown color of the protein concentrate, which makes it undesirable to consumers, and the degradation of proteins leading to a decline in its quality (Hadidi et al., 2019).

Utilization of alfalfa sprouts in human nutrition

Nowadays, there is an urgent need to address the health problems caused by food contamination and malnutrition. These problems are increasingly recognized as a serious worldwide public health concern. Therefore, the production of adequate quantities of safe food for all people worldwide is one of the main issues faced by scientists. Humans mainly depend on food-derived from animal and plant sources, particularly animal and plant-based protein and other protein-based foodstuffs. Due to the previously mentioned problems with animal protein sources, there is an increasing effort to find new, safer, cheaper, and more environmentally friendly plant-protein sources (Gaweł et al., 2017). Sprouts have become an attractive component in the diet of several consumers in many countries due to their high nutritional value (Table 3). These sprouts can be found from more than 20 plant seed species (e.g., alfalfa, mung bean and radish) produced under closed production systems (Fiutak et al,. 2019). The common use of alfalfa sprouts in the human diet includes adding them to mixed fresh vegetables and to salads in order to improve the nutritive value and their palatability (Gaweł et al., 2017). Many studies reported that alfalfa sprouts could be a vital source of protein for human nutrition although there is a link between alfalfa sprouts and food poisoning (e.g., Fiutak et al., 2019; Hong et al., 2019; Lorenzo-Leal et al., 2019; Michalczyk et al., 2019; Mattioli et al., 2019; Mohammad et al., 2019).

The bio-organic fertilizers derived from alfalfa

Within the framework of the green biorefinery, the fresh green or ensiled biomass of alfalfa could be fractionated mechanically into green juice and press cake or solid residues using lactic acid bacteria (Lübeck & Lübeck, 2019). Lactic acid could be produced from the microbial fermentation of the green juice derived from alfalfa. This green juice contains high levels of free amino acids, proteins, fibers, minerals and sugars that could be utilized during microbial fermentation to produce feed additives such as lysine (Thomsen et al., 2015). It is worth to mention that, this green juice may also convert into brown juice if heating at 80 °C or if the lactic acid from fermented green juice is sterilized (Lübeck & Lübeck, 2019). Up to 500 g of brown juice can be produced from 1.0 kg of fresh alfalfa biomass (Manwatkar & Gogle, 2014). Depending on the specific processing techniques, the color of brown juice may vary from yellowish-brown to dark brown or green. This brown juice can be applied as a bio-organic fertilizer to promote crop growth and as a medium for microbial growth (Shende & Gogle, 2016; Bákonyi et al., 2020). The biorefining process of alfalfa has positive impacts not only on the environment but also on human health, which could be expected using novel protein sources (De Corato et al., 2018; Kaszás et al., 2020).

Converting agricultural wastes or the green biomass of alfalfa into practical materials using proper strategies and techniques can be a sustainable approach in the reuse or handling of these resources (Asim et al., 2015). Bio-organic fertilizer can be made by pressing and converting fresh alfalfa biomass into brown liquid. This brown liquid or juice is a by-product produced during the coagulation of alfalfa leaf proteins and fermentation using lactic acid bacteria. Therefore, creation of „bio-organic fertilizer" has received considerable attention over the last decade. This „bio-organic fertilizer" is produced from two components, including organic materials and specific functional microbes (Chen et al., 2019). These organic materials mainly include plant residues (e.g., livestock manure, crop straw, etc.) and pre-treated animal wastes. Recent developments in the production of bio-organic fertilizer have led to a renewed interest in sustainable fertilizer production. These bio-organic fertilizers have benefits such as:

(1) Improving soil quality through removal of pollutants (Chen et al., 2019).

(2) Promoting plant growth by alleviating stress, enhancing the growth of seedlings, and supplying cultivated plants with nutrients (Ansari & Mahmood, 2017; Tahir et al., 2018).

(3) Suppressing soil-borne diseases such as fusarium wilt in banana (Shen et al., 2014;

Zhang et al., 2014), pepper (Wu et al., 2015), cucumber (Huang et al., 2017), watermelon (Liu et al., 2018; Zhao et al., 2018; Xue et al., 2019), and generally acting as a biological control (Ma et al., 2018).

(4) Improving the soil microbial community and enzyme activity (Sun et al., 2016; Jiang et al., 2019; Qu et al., 2019).

(5) Contributing to sustainable development of the agricultural sector through remediating polluted soils and managing agricultural solid wastes (Chen et al., 2019).

There are different kinds of bio-organic fertilizers depending on the source of organic materials and strain or microbial species (Table 4). Alfalfa also has the ability to produce bio-organic fertilizer (Yang et al., 2019b). Further studies are needed to document the different effects of bio-organic fertilizers derived from alfalfa on a variety of cultivated plants under varying environmental conditions.

Conclusion and Future Challenges

Alfalfa is a valuable food and feed crop for humans and animals. It can perform vital functions starting with green biomass and silage production for livestock and sprouts for human consumption. Alfalfa also has the ability to grow and produce desirable yields during drought stress. Moreover, it improves soil fertility when there is lack of nitrogen. Additionally, alfalfa has promising potential as a source of plant-based protein. Moreover, bio-organic fertilizers can be produced during the alfalfa processing and brown juice generation. With respect to a biorefinery based on alfalfa, this crop may be considered as a sustainable solution for the conflict of food and plant-based resources. Therefore, the future challenges of alfalfa may include the following questions: to what extent could alfalfa crop supply the protein demand for human nutrition? When could bio-organic fertilizers derived from alfalfa be produced on a large scale? What are the potential sustainable uses of fiber derived from alfalfa (i.e. generation of energy or nutritive additives)? When will alfalfa gets due consideration as a promising candidate to meet food and energy security demands?

TABLE 3. The chemical composition of alfalfa sprouts compared to alfalfa leaf protein concentrate

Alfalfa sprouts (Fiutak et al., 2019)		Alfalfa leaf protein concentrate (Gaweł et al., 2017)	
Item or component	**Value**	**Item or component**	**Value**
Dry matter of sprouts (g kg^{-1})	67.2	Energy (kcal 100 g^{-1})	344
Dry matter of cotyledons (g kg^{-1})	101	Protein (g kg^{-1})	600
Weight of 100 sprouts (g)	2.25	Fat (g kg^{-1})	225
Mass of cotyledons (g kg^{-1})	424	Carbohydrate (g kg^{-1})	125
Ascorbic acid (mg kg^{-1})	142	Fiber (g kg^{-1})	10
Total phenolic (mg GAE* kg^{-1})	514	Carotene (mg kg^{-1})	867
Chlorophyll *a* (mg kg^{-1} FW)	998.9	Thiamine (mg kg^{-1})	5.0
Chlorophyll *b* (mg kg^{-1} FW)	309.4	Riboflavin (mg kg^{-1})	5.0
β-carotene (mg kg^{-1} FW)	32.3	Niacin (mg kg^{-1})	242
Lutein (mg kg^{-1} FW)	114.0	Folic acid (mg kg^{-1})	0.33
Neoxanthin (mg kg^{-1} FW)	19.0	Ascorbic acid (mg kg^{-1})	22
Violaxanthin (mg kg^{-1} FW)	56.8	Calcium (mg kg^{-1})	18650
		Iron (mg kg^{-1})	990

*GAE: Gallic acid equivalents.

Alfalfa sprouts production under cold LED light.

TABLE 4. Different kinds of bio-organic fertilizers (BOF) based on the source of organic materials and strain or microbial species

Source of organic materials	Microbial strain	The main finding of the study	Reference
Composed of oil rapeseed cakes enzymatically hydrolyzed and pig manure compost (1:1, w/w)	*Bacillus amyloliquefaciens* SQR9	Application of BOF and lime can suppress Fusarium wilt of watermelon and enhance the soil microbial community	Xue et al. (2019)
Prepared by adding biochar, cow manure and poly-γ-glutamic acid	*Arthrobacter sp.* DNS10	BOF may enhance soybean growth and alleviate atrazine stress on the plant	Chen et al. (2019)
Fermented chicken and cow manure compost (1:2, w/w)	*Bacillus amyloliquefaciens* JDF35	Application of BOF may promote plant growth and suppress watermelon Fusarium wilt disease	Zhao et al. (2018)
Prepared by solid fermentation of cattle manure composts	*Bacillus amyloliquefaciens* SQR 9	BOF may suppress Fusarium wilt disease in cucumber and improve soil quality	Huang et al. (2017)
Composed of enzymatically hydrolyzed oil rapeseed cakes and pig manure compost (1:1, w/w)	*Paenibacillus polymyxa* SQR21	Combining BOF with soil fumigant dazomet led to effective control of Fusarium wilt disease in chrysanthemum	Zhao et al. (2016)

Based on the EAT Lancet Commission report (2019), it could be concluded that, the agricultural priorities should be re-oriented from producing high quantities of foods to producing healthy foods. For achieving the UN Sustainable Development Goals, at least half of the losses and wastes of foods should be managed. For safeguarding the universe, global healthy diets should be adopted through sustainable food systems. By 2050, the global consumption of vegetables, fruits, legumes and nuts should be double, whereas the consumption of animal derived foods (like red meat) and sugars should be reduced more than 50 %.

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References

Ansari, R.A., Mahmood, I. (2017) Optimization of organic and bio-organic fertilizers on soil properties and growth of pigeon pea. *Scientia Horticulturae*, **226**, 1-9.

Asim, N., Emdadi, Z., Mohammad, M., Yarmo, M.A., Sopian, K. (2015) Agricultural solid wastes for green desiccant applications: An overview of research achievements, opportunities and perspectives. *Journal of Cleaner Production,* **91**, 26-35.

Bákonyi, N., Kisvarga, S., Barna, D., Tóth, I.O., El-Ramady, H., Abdalla, N., Kovács, S., Rozbach, M., Fehér, C., Elhawat, N., Alshaal, T., Fári, M.G. (2020) Chemical Traits of Fermented Alfalfa Brown Juice: Its Implications on Physiological, Biochemical, Anatomical, and Growth Parameters of Celosia. *Agronomy*, **10**, 247.

Barik, A.K., Hedayetullah, M.D. (2019) Berseem (Egyptian clover). In: "*Forage Crops of the World*", Md. Hedayetullah and P. Zaman (Eds.), pp. 213-230. Volume I: Major Forage Crops. The 1st ed., CRC Press, Apple Academic Press, Inc.

Barros, J., Temple, S., Dixon, R.A. (2019) Development and commercialization of reduced lignin alfalfa. *Current Opinion in Biotechnology*, **56**, 48-54.

Bastidas-Oyanedel Jens, J.-R., Schmidt, E. (2019) Biorefinery: Integrated Sustainable Processes for Biomass Conversion to Biomaterials, Biofuels, and Fertilizers. Springer Nature Switzerland AG, https://doi.org/10.1007/978-3-030-10961-5

Berg, L.E., Miller, S.S., Dornbusch, M.R., Samac, D.A. (2017) Seed rot and damping-off of alfalfa in Minnesota caused by *Pythium* and *Fusarium* species. *Plant Disease*, **101**(11), 1860-1867.

Bessada, S.M.F., Barreira, J.C.M., Oliveira, M.B.P.P. (2019) Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. *Trends in Food Science & Technology*, Doi:10.1016/j.tifs.2019.08.022

Branco, R.H.R., Serafim, L.S., Xavier, A.M.R.B. (2019) Second generation bioethanol production: On the use of pulp and paper industry wastes as feedstock. *Fermentation,* **5**, 4.

Brevik, E.C., Pereg, L., Pereira, P., Steffan, J.J., Burgess, L.C., Gedeon, C.I. (2019) Shelter, clothing, and fuel: often overlooked links between soils, ecosystem services, and human health. *Science of the Total Environment*, **651**, 134-142.

Broderick, G.A. (2018) Utilization of protein in red clover and alfalfa silages by lactating dairy cows and growing lambs. *Journal of Dairy Science,* **101**(2), 1190-1205.

Chen, H., Li, L., Luo, X., Li, Y., Deng, J. (2019) Modeling impacts of mulching and climate change on crop production and N_2O emission in the Loess Plateau of China. *Agricultural and Forest Meteorology,* **268**, 86-97.

Contreras, M.D.M., Lama-Muñoz, A., Manuel Gutiérrez-Pérez, J., Espínola, F., Moya, M., Castro, E. (2019) Protein extraction from agri-food residues for integration in biorefinery: Potential techniques and current status. *Bioresource Technology*, **280**, 459-477.

Cui, Y., Liu, D., Chen, J. (2018) Fate of various *Salmonella enterica* and enterohemorrhagic Escherichia coli cells attached to alfalfa, fenugreek, lettuce, and tomato seeds during germination. *Food Control*, **88**, 229–235.

Dal Bosco, A., Castellini, C., Martino, M., Mattioli, S., Marconi, O., Sileoni, V., Ruggeri, S., Tei, F., Benincasa, P. (2015) The effect of dietary alfalfa and flax sprouts on rabbit meat antioxidant content, lipid oxidation and fatty acid composition. *Meat Science,* **106**, 31-7.

De Bhowmick, G., Sarmah, A.K., Sen, R. (2018) Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products. *Bioresource Technology*, **247**, 1144-1154.

De Corato, U., De Bari, I., Viola, E., Pugliese, M. (2018) Assessing the main opportunities of integrated biorefining from agro-bioenergy co/by-products and agroindustrial residues into high-value added products associated to some emerging markets: A review. *Renewable and Sustainable Energy Reviews,* **88**, 26–346.

Dietz, D., Schneider, R., Papendiek, F., Venus, J. (2016) Leguminose green juice as an efficient nutrient for l(+)-lactic acid production. *Journal of Biotechnology,* **236**, 26-34.

Dong, Z., Yuan, X., Wen, A., Desta, S.T., Shao, T. (2017) Effects of calcium propionate on the fermentation quality and aerobic stability of alfalfa silage. *Asian-Australasian Journal of Animal Sciences,* **30**(9), 1278-1284.

EAT Lancet Commission report (2019) Extarcted from the following link on 28 May 2020: https://eatforum.org/content/uploads/2019/07/EAT-Lancet_Commission_Summary_Report.pdf

Fan, W., Ge, G., Liu, Y., Wang, W., Liu, L., Jia ,Y. (2018) Proteomics integrated with metabolomics: analysis of the internal causes of nutrient changes in alfalfa at different growth stages. *BMC Plant Biology,* **18**(1), 78.

FAO (2019) Alfalfa. Accessed on 25 August 2019 on the link http://www.fao.org/land-water/databasesand-software/crop-information/alfalfa/en/

Fiutak, G., Michalczyk, M., Filipczak-Fiutak, M., Fiedor, L., Surówka, K. (2019) The impact of LED lighting on the yield, morphological structure and some bioactive components in alfalfa (*Medicago sativa* L.) sprouts. *Food Chemistry,* **285**, 53-58.

Fustini, M., Palmonari, A., Canestrari, G., Bonfante, E., Mammi, L., Pacchioli, M.T., Sniffen, G.C.J., Grant, R.J., Cotanch, K.W., Formigoni, A. (2017) Effect of undigested neutral detergent fiber content of alfalfa hay on lactating dairy cows: Feeding behavior, fiber digestibility, and lactation performance. *Journal of Dairy Science,* **100**(6), 4475-4483.

Gaafar, Y.Z.A., Richert-Pöggeler, K.R., Maaß, C., Vetten, H.J., Ziebell, H. (2019) Characterisation of a novel nucleorhabdovirus infecting alfalfa (*Medicago sativa*). *Virology Journal,* **16**(1), 55.

Gallego-Giraldo, L., Shadle, G., Shen, H., Barros-Rios, J., Fresquet Corrales, S., Wang, H., Dixon, R.A. (2016) Combining enhanced biomass density with reduced lignin level for improved forage quality. *Plant Biotechnology Journal,* **14**(3), 895-904.

Gao, R., Gruber, M.Y., Amyot, L., Hannoufa, A. (2018) SPL13 regulates shoot branching and flowering time in *Medicago sativa*. *Plant Molecular Biology,* **96**(1-2), 119-133.

Gardner, C.D., Hartle, J.C., Garrett, R.D., Offringa, L.C., Wasserman, A.S. (2019) Maximizing the intersection of human health and the health of the environment with regard to the amount and type of protein produced and consumed in the United States. *Nutrition Reviews,* **77**(4), 197-215.

Gaweł, E. (2012) Chemical composition of lucerne leaf extract (EFL) and its applications as a phytobiotic in human nutrition. *Acta Scientiarum Polonorum Technologia Alimentaria,* **11**(3), 303-10.

Gaweł, E., Grzelak, M., Janyszek, M. (2017) Lucerne (*Medicago sativa* L.) in the human diet—Case reports and short reports. *Journal of Herbal Medicine,* **10**, 8–16.

Getachew, G., Laca, E.A., Putnam, D.H., Witte, D., McCaslin, M., Ortega, K.P., DePeters, E.J. (2018) The impact of lignin down regulation on alfalfa yield, chemical composition, and *in vitro* gas production. *Journal of the Science of Food and Agriculture,* **98**(11), 4205-4215.

Ghaderpour, O., Rafiee, S., Sharifi, M., Mousavi-Avval, S.H. (2018) Quantifying the environmental impacts of alfalfa production in different farming systems. *Sustainable Energy Technologies and Assessments,* **27**, 109–118.

Gou, J., Debnath, S., Sun, L., Flanagan, A., Tang, Y., Jiang, Q., Wen, J., Wang, Z.Y. (2018) From model to crop: functional characterization of SPL8 in *M. Truncatula* led to genetic improvement of biomass yield and abiotic stress tolerance in alfalfa. *Plant Biotechnology Journal,* **16**(4), 951-962.

Gruber, M.Y., Xia, J., Yu, M., Steppuhn, H., Wall, K., Messer, D., Sharpe, A.G., Acharya, S.N., Wishart, D.S., Johnson, D., Miller, D.R., Taheri, A. (2017) Transcript analysis in two alfalfa salt tolerance selected breeding populations relative to a non-tolerant population. *Genome,* **60**(2), 104-127.

Guo, G., Shen, C., Liu, Q., Zhang, S.L., Wang, C., Chen, L., Xu, Q.F., Wang, Y.X., Huo, W.J. (2019) Fermentation quality and *in vitro* digestibility of first and second cut alfalfa (*Medicago sativa* L.) silages harvested at three stages of maturity. *Animal Feed Science and Technology,* 114274. doi:10.1016/j.anifeedsci.2019.114274

Hadidi, M., Ibarz, A., Conde, J., Pagan, J. (2019) Optimisation of steam blanching on enzymatic activity, color and protein degradation of alfalfa (*Medicago sativa*) to improve some quality characteristics of its edible protein. *Food Chemistry,*. **276**, 591-598.

Hartinger, T., Gresner, N., Südekum, K.-H. (2019) Effect of wilting intensity, dry matter content and sugar addition on nitrogen fractions in Lucerne silages. *Agriculture,* **9**, 11.

Hassan, S.S., Williams, G.A., Jaiswal, A.K. (2019) Moving towards the second generation of lignocellulosic biorefineries in the EU: Drivers, challenges, and opportunities. *Renewable and Sustainable Energy Reviews*, **101**, 590-599.

Hassen, A., Talore, D.G., Tesfamariam, E.H., Friend, M.A., Mpanza, T.D.E. (2017) Potential use of forage-legume intercropping technologies to adapt to climate-change impacts on mixed crop-livestock systems in Africa: a review. *Regional Environmental Change*, **17**(6), 1713–1724.

Hawkins, C., Yu, L.-X. (2018) Recent progress in alfalfa (*Medicago sativa* L.) genomics and genomic selection. *Crop Journal*, **6** (6), 565-575.

Hojilla-Evangelista, M.P., Selling, G.W., Hatfield, R., Digman, M. (2017) Extraction, composition, and functional properties of dried alfalfa (*Medicago sativa* L.) leaf protein. *Journal of the Science of Food and Agriculture,* **97**(3), 882-888.

Hong, E.-J., Park, S.-H., Kang, D.-H. (2019) Sequential treatment of hydrogen peroxide, vacuum packaging, and dry heat for inactivating *Salmonella typhimurium* on alfalfa seeds without detrimental effect on seeds viability. *Food Microbiology,* doi:10.1016/j.fm.2018.09.002

Huang, N., Wang, W., Yao, Y., Zhu, F., Wang, W., Chang, X. (2017) The influence of different concentrations of bio-organic fertilizer on cucumber Fusarium wilt and soil microflora alterations. *PloS One*, **12**(2), e0171490.

Jia, C., Wu, X., Chen, M., Wang, Y., Liu, X., Gong, P., Xu, Q., Wang, X., Gao, H., Wang, Z. (2017) Identification of genetic loci associated with crude protein and mineral concentrations in alfalfa (*Medicago sativa*) using association mapping. *BMC Plant Biology,* **17**(1), 97.

Jiang, S.Q., Yu, Y.N., Gao, R.W., Wang, H., Zhang, J., Li, R., Long, X.H., Shen, Q.R., Chen, W., Cai, F. (2019) High-throughput absolute quantification sequencing reveals the effect of different fertilizer applications on bacterial community in a tomato cultivated coastal saline soil. *Science of the Total Environment,* **687**, 601-609.

Jonker, A., Hickey, S., Pinares-Patiño, C., McEwan, J., Olinga, S., Díaz, A., Molano, G., MacLean, S., Sandoval, E., Harland, R., Birch, D., Bryson, B., Knowler, K., Rowe, S. (2017) Sheep from low-methane-yield selection lines created on alfalfa pellets also have lower methane yield under pastoral farming conditions. *Journal of Animal Science,* **95**(9), 3905-3913.

Jonker, A., Yu, P. (2016) The Role of Proanthocyanidins Complex in Structure and Nutrition Interaction in Alfalfa Forage. *International Journal of Molecular Sciences*, **17**(5), 793.

Jozefkowicz, C., Frare, R., Fox, R., Odorizzi, A., Arolfo, V., Pagano, E., Basigalup, D., Ayub, N., Soto, G. (2018) Maximizing the expression of transgenic traits into elite alfalfa germplasm using a supertransgene configuration in heterozygous conditions. *Theoretical and Applied Genetics,* **131**(5), 1111-1123.

Ju, W., Liu, L., Fang, L., Cui, Y., Duan, C., Wu, H. (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. *Ecotoxicology and Environmental Safety,* **167**, 218-226.

Kang, J.-W., Lee, J.-I., Jeong, S.-Y., Kim, Y.-M., Kang, D.-H. (2019) Effect of 222-nm krypton-chloride excilamp treatment on inactivation of *Escherichia coli* O157:H7 and *Salmonella Typhimurium* on alfalfa seeds and seed germination. *Food Microbiology*, **82**, 171–176.

Kaszás, L., Alshaal, T., Kovács, Z., Koroknai, J., Elhawat, N., Nagy, É., El-Ramady, H., Fári, M., Domokos-Szabolcsy, É. (2020) Refining high-quality leaf protein and valuable co-products from green biomass of Jerusalem artichoke (*Helianthus tuberosus* L.) for sustainable protein supply. *Biomass Conversion and Biorefinery*, https://doi.org/10.1007/s13399-020-00696-z

Kavut, Y.T., Avcioglu, R. (2015) Yield and quality performances of various alfalfa (*Medicago sativa* L.) cultivars in different soil textures in a Mediterranean environment. *Turkish Journal of Field Crops,* **20**(1), 65-71.

Ke, W.C., Ding, W.R., Xu, D.M., Ding, L.M., Zhang, P., Li, F.D., Guo, X.S. (2017) Effects of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage. *Journal of Dairy Science*, **100**(11), 8958-8966.

Ke, W.C., Ding, W., Xu, D., Shah, M.N., Zhang, P., Guo, X. (2018a) Influences of addition of malic acid or citric acid, *Lactobacillus plantarum* and their mixtures on fermentation quality, proteolysis and fatty acid composition of ensiled alfalfa. *Archives of Animal Nutrition,* **72**(6), 492-502.

Ke, W.C., Ding, W.R., Ding, L.M., Xu, D.M., Zhang, P., Li, F.H., Guo, X.S. (2018b) Influences of malic acid isomers and their application levels on fermentation quality and biochemical characteristics of alfalfa silage. *Animal Feed Science and Technology,* doi:10.1016/j.anifeedsci.2018.08.012

Keshri, J., Krouptiski, Y., Abu-Fani, L., Achmon, Y., Bauer, T.S., Zarka, O., Maler, I., Pinto, R., Sela Saldinger, S. (2019) Dynamics of bacterial communities in alfalfa and mung bean sprouts during refrigerated conditions. *Food Microbiology,* **84**, 103261. doi: 10.1016/j.fm.2019.103261.

Khan, K., Khan, S., Khan, R., Sultan, A., Khan, N.A., Ahmad, N. (2016) Growth performance and meat quality of rabbits under different feeding regimes. *Tropical Animal Health and Production,* **48**(8), 1661-1666.

Kim, W.-I., Ryu, S.D., Kim, S.-R., Kim, H.-J., Lee, S., Kim, J. (2018) Population changes and growth modeling of *Salmonella enterica* during alfalfa seed germination and early sprout development. *Food Science and Biotechnology,* doi:10.1007/s10068-018-0412-3

Kulkarni, K.P., Tayade, R., Asekova, S., Song, J.T., Shannon, J.G., Lee, J.D. (2018) Harnessing the potential of forage legumes, alfalfa, soybean, and cowpea for sustainable agriculture and global food security. *Frontiers in Plant Science,* **9**, 1314. doi: 10.3389/fpls.2018.01314.

Lai, L.J., Chiu, J.M., Chiou, R.Y. (2017) Fresh preservation of alfalfa sprouts and mushroom slices by soaking with thymol and resveratrol solutions. *Food Science & Nutrition,* **5**(3), 776-783.

Lamsal, B.P., Koegel, R.G., Gunasekaran, S. (2007) Some physicochemical and functional properties of alfalfa soluble leaf proteins. *LWT - Food Science and Technology,* **40**, 1520–1526.

Lee, M.A. (2018) A global comparison of the nutritive values of forage plants grown in contrasting environments. *Journal of Plant Research,* **131**(4), 641-654.

Lei, Y., Hannoufa, A., Yu, P. (2017) The use of gene modification and advanced molecular structure analyses towards improving alfalfa forage. *International Journal of Molecular Sciences,* **18**(2). pii: E298. doi: 10.3390/ijms18020298.

Lei, Y., Xu, Y., Hettenhausen, C., Lu, C., Shen, G., Zhang, C., Li, J., Song, J., Lin, H., Wu, J. (2018) Comparative analysis of alfalfa (*Medicago sativa* L.) leaf transcriptomes reveals genotype-specific salt tolerance mechanisms. *BMC Plant Biology,* **18**(1), 35. doi: 10.1186/s12870-018-1250-4.

Li, D.-X., Ni, K., Zhang, Y., Lin, Y., Yang, F. (2018) Influence of lactic acid bacteria, cellulase, cellulase-producing *Bacillus pumilus* and their combinations on alfalfa silage quality. *Journal of Integrative Agriculture,* **17**(12), 2768-2782.

Li, Y., Duan, T., Nan, Z., Li, Y. (2019) Arbuscular mycorrhizal fungus alleviates alfalfa leaf spots caused by Phoma medicaginis revealed by RNA-seq analysis. *Journal of Applied Microbiology*, doi: 10.1111/jam.14387.

Liu, H.-A., Rodrigo-Comino, J., Wu, H.-S., Yang, G.-Y., Ma, X.-L., Wang, X.-J., Chen, K.-K., Liu, Y.-D., Brevik, E.C. (2018) Assessment of a new bio-organic remediation as a biofungicide in *fusarium*-infested soils of watermelon monoculture areas from China. *Journal of Soil Science and Plant Nutritionis,* **18**(3), 735-751.

Longato, D., Gaglio, M., Boschetti, M., Gissi, E. (2019) Bioenergy and ecosystem services trade-offs and synergies in marginal agricultural lands: A remote-sensing-based assessment method. *Journal of Cleaner Production,* **237**, Article 117672.

Lonnie, M., Hooker, E., Brunstrom, J.M., Corfe, B.M., Green, M.A., Watson, A.W., Williams, E.A., Stevenson, E.J., Penson, S., Johnstone, A.M. (2018) Protein for life: Review of optimal protein intake, sustainable dietary sources and the effect on appetite in ageing adults. *Nutrients*, **10**(3). pii: E360. doi: 10.3390/nu10030360.

Lorenzo-Leal, A.C., Palou, E., López-Malo, A. (2019) Evaluation of the efficiency of allspice, thyme and rosemary essential oils on two foodborne pathogens in in-vitro and on alfalfa seeds, and their effect on sensory characteristics of the sprouts. *International Journal of Food Microbiology*, **295**, 19-24.

Luo, D., Zhou, Q., Wu, Y., Chai, X., Liu, W., Wang, Y., Yang, Q., Wang, Z., Liu, Z. (2019) Full-length transcript sequencing and comparative transcriptomic analysis to evaluate the contribution of osmotic and ionic stress components towards salinity tolerance in the roots of cultivated alfalfa (*Medicago sativa* L.). *BMC Plant Biology,* **19**(1), 32. doi: 10.1186/s12870-019-1630-4.

Luo, J., Balvert, S.F., Wise, B., Welten, B., Ledgard, S.F., de Klein, C.A.M., Lindsey, S., Judge, A. (2018) Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. *Science of the Total Environment*, **610-611**, 1271–1280.

Lübeck, M., Lübeck, P.S. (2019) Application of lactic acid bacteria in green biorefineries. *FEMS Microbiology Letters,* **366**(3). Pii: fnz024. Doi: 10.1093/femsle/fnz024.

Ma, L., Zhang, H.-Y., Zhou, X.-K., Yang, C.-G., Zheng, S.-C., Duo, J.-L., Mo, M.-H. (2018) Biological control tobacco bacterial wilt and black shank and root colonization by bio-organic fertilizer containing bacterium *Pseudomonas aeruginosa* NXHG29. *Applied Soil Ecology,* **129**, 136-144.

Manwatkar, V.G., Gogle, D.P. (2014) The effect of deproteinised juice (DPJ) on seed germination and seedling growth of different plants (by paper towel method). *International Journal of Life Sciences,* **Special Issue** A2, 65-68.

Mattioli, S., Dal Bosco, A., Castellini, C., Falcinelli, B., Sileoni, V., Marconi, O., Mancinelli, A.C., Cotozzolo, E., Benincasa, P. (2019) Effect of heat- and freeze-drying treatments on phytochemical content and fatty acid profile of alfalfa and flax sprouts. *Journal of the Science of Food and Agriculture*, **99**(8), 4029-4035.

Mbarki S, Cerdà A, Zivcak, M., Brestic, M., Rabhi, M., Mezni, M., Jedidi, N., Abdelly, C., Pascual, J.A. (2018) Alfalfa crops amended with MSW compost can compensate the effect of salty water irrigation depending on the soil texture. *Process Safety and Environmental Protection,* **115**, 8–16.

Michalczyk, M., Fiutak, G., Tarko, T. (2019) Effect of hot water treatment of seeds on quality indicators of alfalfa sprouts. *LWT- Food Science and Technology*, **113**, 108270. doi:10.1016/j.lwt.2019.108270

Mohammad, Z., Kalbasi-Ashtari, A., Riskowski, G., Castillo, A. (2019) Reduction of *Salmonella* and *Shiga* toxin-producing *Escherichia coli* on alfalfa seeds and sprouts using an ozone generating system. *International Journal of Food Microbiology,* **289**, 57-63. doi: 10.1016/j.ijfoodmicro.2018.08.023.

Monteros, M.J., Yhan, E., Brummer, C. (2014) Alfalfa. In: "*Genetics, Genomics and Breeding of Forage Crops*", H. Cai, T. Yamada and C. Kole (Eds.), pp. 187 – 219. CRC Press, Taylor & Francis Group, LLC.

Morin, É., Michaud-Létourneau, I., Couturier, Y., Roy, M. (2019) A whole-food, plant-based nutrition program: Evaluation of cardiovascular outcomes and exploration of food choices determinants. *Nutrition,* **66**, 54-61.

Nemchinov, L.G., Shao, J., Lee, M.N., Postnikova, O.A., Samac, D.A. (2017) Resistant and susceptible responses in alfalfa (*Medicago sativa*) to bacterial stem blight caused by *Pseudomonas syringae* pv. Syringae. *PloS One,* **12**(12), e0189781. doi: 10.1371/journal.pone.0189781.

Ogunade, I.M., Jiang, Y., Pech Cervantes, A.A., Kim, D.H., Oliveira, A.S., Vyas, D., Weinberg, Z.G., Jeong, K.C., Adesogan, A.T. (2018) Bacterial diversity and composition of alfalfa silage as analyzed by Illumina MiSeq sequencing: Effects of *Escherichia coli* O157:H7 and silage additives*. Journal of Dairy Science,* **101**(3), 2048–2059.

Palmonari, A., Fustini, M., Canestrari, G., Grilli, E., Formigoni, A. (2014) Influence of maturity on alfalfa hay nutritional fractions and indigestible fiber content. *Journal of Dairy Science,* **97**(12), 7729-34.

Papendiek, F. (2017) Fodder legumes for green biorefineries: A perspective for sustainable agricultural production systems. In: "*Food Waste Reduction and Valorisation*", P. Morone et al. (Eds.), pp. 69-78. doi: 10.1007/978-3-319-50088-1_5, Springer International Publishing AG.

Papendiek, F., Tartiu, V.E., Morone, P., Venus, J., Hönig, A. (2016) Assessing the economic profitability of fodder legume production for Green Biorefineries – A cost-benefit analysis to evaluate farmers profitability. *Journal of Cleaner Production,* **112**, Part 5, 3643-3656.

Patel, H., Chandra, S., Alexander, S., Soble, J., Williams, K.A. (2017) Plant-based nutrition: An essential component of cardiovascular disease prevention and management. *Current Cardiology Reports*, **19**(10), 104.

Patra, P.S., Paul, T. (2019) Lucerne (Alfalfa). In: "*Forage Crops of the World*", Md. Hedayetullah and P. Zaman (Eds.), pp. 231-243. Volume I: Major Forage Crops. The 1st edition, CRC Press, Apple. Academic Press, Inc.

Qu, C.C., Chen, X.M., Zhang, Z.L., Wang, N., Lyu, J.Y., Zhang, J., Huang, C.Y. (2019) Long-term effects of bio-organic fertilizer application on soil organic carbon pool and enzyme activity of cucumber continuous cropping. *Ying yong sheng tai xue bao*, **30**(9), 3145-3154 (in Chinese).

Rajabi, R., Tahmasbi, R., Dayani, O., Khezri, A. (2017) Chemical composition of alfalfa silage with waste date and its feeding effect on ruminal fermentation characteristics and microbial protein synthesis in sheep. *Journal of Animal Physiology and Animal Nutrition (Berl),* **101**(3), 466-474.

Richter, C.K., Skulas-Ray, A.C., Champagne, C.M., Kris-Etherton, P.M. (2015) Plant protein and animal proteins: Do they differentially affect cardiovascular disease risk? *Advances in Nutrition,* **6**(6), 712–728.

Sah, R.P., Kumar, A., Rana, M., Kumar, U. (2019) Maize (Corn). In: "*Forage Crops of the World*", Md. Hedayetullah and P. Zaman (Eds.), pp. 15-37. Volume I: Major Forage Crops. The 1st ed., CRC Press, Apple. Academic Press, Inc.

Samac, D.A., Schraber, S., Barclay, S. (2015) A mineral seed coating for control of seedling diseases of alfalfa suitable for organic production systems. *Plant Disease,* **99**(5), 614-620.

Samarfard, S., Bejerman, N.E., Dietzgen, R.G. (2018) Distribution and genetic variability of alfalfa dwarf virus, a cytorhabdovirus associated with alfalfa dwarf disease in Argentina. *Virus Genes,* **54**(4), 612-615.

Santamaría-Fernández, M., Molinuevo-Salces, B., Lübeck, M., Uellendahl, H. (2018) Biogas potential of green biomass after protein extraction in an organic biorefinery concept for feed, fuel and fertilizer production. *Renewable Energy,* **129**, Part B, 769-775.

Santos, M.C., Kung, L. (2016) Short communication: The effects of dry matter and length of storage on the composition and nutritive value of alfalfa silage. *Journal of Dairy Science,* **99**(7), 5466–5469.

Sarnataro, C., Petri, R.M., Spanghero, M., Zebeli, Q., Klevenhusen, F. (2019) A nutritional and rumen ecological evaluation of the biorefinery by-product alfalfa silage cake supplemented with *Scrophularia striata* extract using the rumen simulation technique. *Journal of the Science of Food and Agriculture,* **99**(9), 4414-4422.

Shen, Z., Wang, D., Ruan, Y., Xue, C., Zhang, J., Li, R., Shen, Q. (2014) Deep 16S rRNA pyrosequencing reveals a bacterial community associated with Banana *Fusarium* Wilt disease suppression induced by bio-organic fertilizer application. *PloS One,* **9**(5), e98420.

Shende, G.C., Gogle, D.P. (2016) To study the effect of various concentration of deproteinized leaf juice (DPJ) of selected plants on growth of *Aspergillus niger*. *International Journal of Life Sciences*, **A6**, 186-188.

Shi, S., Nan, L., Smith, K.F. (2017) The current status, problems, and prospects of alfalfa (*Medicago sativa* L.) breeding in China. *Agronomy,* **7**, 1. doi:10.3390/agronomy7010001

Singer, S.D., Hannoufa, A., Acharya, S. (2018) Molecular improvement of alfalfa for enhanced productivity and adaptability in a changing environment. *Plant, Cell & Environment,* **41**(9), 1955-1971.

Sisterson, M.S., Dwyer, D.P., Uchima, S.Y. (2018) Alfalfa and Pastures: Sources of Pests or Generalist Natural Enemies? *Environmental Entomology,* **47**(2), 271-281.

Srivastava, N., Srivastava, M., Mishra, P.K., Upadhyay, S.N., Ramteke, P.W., Gupta, V.K. (2019) Sustainable approaches for biofuels production technologies: From current status to practical implementation. *Biofuels, Bioproducts and Biorefining*, **7**, Springer International Publishing AG, https://doi.org/10.1007/978-3-319-94797-6.

Sun, J.U., Fu, Q.X., Gu, J., Wang, X.J., Gao. H. (2016) Effects of bio-organic fertilizer on soil enzyme activities and microbial community in kiwifruit orchard. *Ying Yong Sheng Tai Xue Bao*, **27**(3), 829-837 (in Chinese).

Tahir, M., Khalid, U., Ijaz, M., Shah, G.M., Naeem, M.A., Shahid, M., Mahmood, K., Ahmad, N., Kareem, F. (2018) Combined application of bio-organic phosphate and phosphorus solubilizing bacteria (*Bacillus strain* MWT 14) improve the performance of bread wheat with low fertilizer input under an arid climate. *Brazilian Journal of Microbiology,* **49 Suppl 1**, 15-24.

Tao, L., Zhou, H., Zhang, N., Si, B., Tu, Y., Ma, T., Diao, Q. (2017) Effects of different source additives and wilt conditions on the pH value, aerobic stability, and carbohydrate and protein fractions of alfalfa silage. *Animal Science Journal,* **88**(1), 99-106.

Thomsen, M.H., Alassali, A., Cybulska, I., Yousef, A.F., Brown, J.J., Andersen, M., Ratkov, A., Kiel, P. (2015) Microorganisms for biorefining of green biomass. In: "*Microorganisms in Biorefineries. Microbiology Monographs*", Kamm, B. (Ed.), 26, pp. 157-181. Springer, Berlin, Heidelberg.

Tian, J., Li, Z., Yu, Z., Zhang, Q., Li, X. (2017) Interactive effect of inoculant and dried jujube powder on the fermentation quality and nitrogen fraction of alfalfa silage. *Animal Science Journal,* **88**(4), 633-642.

Turan, A., Önenç, S.S. (2018) Effect of cumin essential oil usage on fermentation quality, aerobic stability and in vitro lfalfaility of alfalfa silage. *Asian-Australasian Journal of Animal Sciences,* **31**(8), 1252-1258.

Vintu, V., Stavarache, M., Samuil, C., Munteanu, I. (2012) Chemical composition dynamics of alfalfa (*Medicago sativa* L.) at different plant growth stages. *Conference: Proceedings of the 24th General Meeting of the European Grassland Federation Lublin,* Poland 3–7 June 2012 At: Lublin, Poland Volume: 17. Grassland Science in Europe

Wang, C., He, L., Xing, Y., Zhou, W., Yang, F., Chen, X., Zhang, Q. (2019) Fermentation quality and microbial community of alfalfa and stylo silage mixed with *Moringa oleifera* leaves. *Bioresource Technology,* **284**, 240-247.

Wang, S., Jiao, X., Guo, W., Lu, J., Bai, Y., Wang, L. (2018) Adaptability of shallow subsurface drip irrigation of alfalfa in an arid desert area of Northern Xinjiang. *PloS One,* **13**(4), e0195965. doi: 10.1371/journal.pone.0195965

Wang, Z., Qiang, H., Zhao, H., Xu, R., Zhang, Z., Gao, H., Wang, X., Liu, G., Zhang, Y. (2016) Association mapping for fiber-related traits and digestibility in alfalfa (*Medicago sativa*). *Frontiers in Plant Science,* **7**, 331. doi: 10.3389/fpls.2016.00331.

Wen, C., Zhang, J., Yao, H., Zhou, J., Duan, Y., Zhang, H., Ma, H. (2019) Advances in renewable plant-derived protein source: The structure, physicochemical properties affected by ultrasonication. *Ultrasonics Sonochemistry,* Doi:10.1016/j.ultsonch.2018.12.036

Wu, Y., Zhao, C., Farmer, J., Sun, J. (2015) Effects of bio-organic fertilizer on pepper growth and Fusarium wilt biocontrol. *Scientia Horticulturae,* **193**, 114-120.

Xiang, Q., Liu, X., Liu, S., Ma, Y., Xu, C., Bai, Y. (2019) Effect of plasma-activated water on microbial quality and physicochemical characteristics of mung bean sprouts. *Innov. Food Sci. Emerging Technologies*, doi:10.1016/j.ifset.2018.11.012

Xu, S., Christensen, M.J., Creamer, R., Li, Y.Z. (2019) Identification, characterization, pathogenicity, and distribution of *Verticillium lfalfa* in alfalfa plants in China. *Plant Disease,* **103**(7), 1565-1576.

Xue, C., Shen, Z., Hao, Y., Yu, S., Li, Y., Huang, W., Chong, Y., Ran, W., Li, R., Shen, Q. (2019) Fumigation coupled with bio-organic fertilizer for the suppression of watermelon Fusarium wilt disease re-shapes the soil microbiome. *Applied Soil Ecology,* **140**, 49-56.

Yang, H., An, F., Yang, F., Wang, Z. (2019a) The impact of irrigation on yield of alfalfa and soil chemical properties of saline-sodic soils. *PeerJ,* **7**, e7148. doi: 10.7717/peerj.7148.

Yang, L., Yuan, X., Li, J., Dong, Z., Shao, T. (2019b) Dynamics of microbial community and fermentation quality during ensiling of sterile and nonsterile alfalfa with or without *Lactobacillus plantarum* inoculant. *Bioresource Technology,* **275**, 280-287.

Yang, Y.Y., Gao, Z.L., Wang, X.J. (2016) Impacts of organic and inorganic fertilizations on alfalfa yield, soil nitrate and greenhouse gas emissions. *Ying Yong Sheng Tai Xue Bao,* **27**(3), 822-828 (in Chinese).

Yuan, X., Wen, A., Desta, S.T., Wang, J., Shao, T. (2017) Effects of sodium diacetate on the fermentation profile, chemical composition and aerobic stability of alfalfa silage. *Asian Australasian Journal of Animal Sciences,* **30**(6), 804-810.

Yuan, X.J., Wen, A.Y., Wang, J., Desta, S.T., Dong, Z.H., Shao, T. (2018) Effects of four short-chain fatty acids or salts on fermentation characteristics and aerobic stability of alfalfa (*Medicago sativa* L.) silage. *Journal of the Science of Food and Agriculture,* **98**(1), 328-335.

Zeng, N., Yang, Z., Zhang, Z., Hu, L., Chen, L. (2019) Comparative transcriptome combined with proteome analyses revealed key factors involved in alfalfa (*Medicago sativa*) response to waterlogging stress. *International Journal of Molecular Sciences,* **20**(6). Pii: E1359. doi: 10.3390/ijms20061359.

Zhang, F., Kang, J., Long, R., Yu, L.X., Wang, Z., Zhao, Z., Zhang, T., Yang, Q. (2019) High-density linkage map construction and mapping QTL for yield and yield components in autotetraploid alfalfa using RAD-seq. *BMC Plant Biology,* **19**(1), 165. doi: 10.1186/s12870-019-1770-6.

Zhang, N., He, X., Zhang, J., Raza, W., Shen, R., Yang, X.-M., Ruan, Y.-Z., Huang, Q.-W. (2014) Suppression of Fusarium Wilt of Banana with Application of Bio-Organic Fertilizers. *Pedosphere,* **24** (5), 613-624.

Zhang, Q., Yu, Z., Wang, X., Na, R. (2017) Effects of chlorpyrifos and chlorantraniliprole on fermentation quality of alfalfa (*Medicago sativa* L.) silage inoculated with or without *Lactobacillus plantarum* LP. *Animal Science Journal*, **88**(3), 456-462.

Zhao, J., Wang, Y., Liang, H., Huang, J., Chen, Z., Nie, Y. (2018) The rhizosphere microbial community response to a bio-organic fertilizer: finding the mechanisms behind the suppression of watermelon Fusarium wilt disease. *Acta Physiologiae Plantarum,* **40**, 17. https://doi.org/10.1007/s11738-017-2581-8

Zhao, S., Chen, X., Deng, S., Dong, X., Song, A., Yao, J., Fang, W., Chen, F. (2016) The effects of fungicide, soil fumigant, bio-organic fertilizer and their combined application on *Chrysanthemum* Fusarium Wilt controlling, soil enzyme activities and microbial properties. *Molecules,* **21**(4), 526. doi: 10.3390/molecules21040526.

التكرير الحيوى المستدام لنبات البرسيم الحجازى: بحث مرجعى

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يعتبر البرسيم الحجازى «ملكة الأعلاف» نظرا لقيمته الغذائية وإرتفاع محتواه من البروتين. إن إنتاج البرسيم الحجازى تحت ظروف الإجهادات البيئية المختلفة، والتي تمثل تهديدًا خطيرًا للأمن الغذائي العالمي، تحديًا كبيرًا. ولتحسين إنتاجية البرسيم الحجازى تحت ظروف الإجهاد ، فإن هناك حاجة ماسة لفهم إستجابة نباتات البرسيم الحجازى للإجهاد وآليات التحمل وأساليب الإدارة. البرسيم الحجازى محصول حيوى واعد، فمنه يمكن إنتاج مركزات بروتين الأوراق والأسمدة العضوية الحيوية. إن إمكانات تركيز البروتين المشتق من الأوراق لتغذية الإنسان والحيوان هائلة، ولكنها غير مستغلة. تمثل الأسمدة العضوية الحيوية المشتقة من نباتات البرسيم الحجازى طريقة مستدامة لتزويد النبات ببعض المغذيات. تسلط هذه المقالة المرجعية الضوء على الإستخدام المستدام للبرسيم الحجازى في التكرير الحيوى وإنتاج الأسمدة العضوية الحيوية. علاوة على ذلك، يتم جمع المعلومات المتناثرة حول الإستفادة من إنتاج البرسيم الحجازى ودعم مدى ملاءمته لتحسين خصوبة التربة. أخيرًا، نناقش مدى ملاءمة البرسيم لإنتاج غلات مستقرة في ضوء تغير المناخ وظروف الإجهاد مثل الملوحة.