Scientific Papers. Series D. Animal Science. Vol. LXII, No. 1, 2019
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CHEMICAL AND NUTRITIONAL PROPERTIES OF POTENTIAL PHYTOADDITIVES USED IN ANIMAL NUTRITION

Alexandra LUPU, Margareta OLTEANU, Arabela UNTEA, Iulia VĂRZARU, Mariana ROPOTĂ

National Institute of Research-Development for Biology and Animal Nutrition - IBNA Balotesti, Romania

Corresponding author email: alexandra.lupu@ibna.ro

Abstract

Phytoadditives are used in animal nutrition for prevention and treatment of potential disease, as antioxidants and digestion stimulants. Three medicinal plants: mint (Mentha longifolia), fennel (Foeniculum vulgare) and white willow bark (Salix alba)) were analysed for their proximate composition, minerals, fatty acids and amino acids concentrations. The results revealed an important concentration of crude protein in menthe and fennel (20.72% and 18.89%), while fennel seeds have significant quantities of crude fiber (27.31%). White willow bark have a low content in crude protein (0.18%) and in crude fat (0.01%). Mint is good source of fatty acids with significant quantities of ω3 and a ω3/ω6 ratio below 1. The medicinal plants investigated are good sources of minerals, higher concentrations was found in menthe regarding concentration in Fe (1408.98 ppm), Mn (106.91 ppm) and Ca (1.50%). The results indicated important values of essential amino acids in mint and fennel (9.036 and 7.655 g/100g DM) and semi-essential amino acids (2.891 and 2.986 g/100 g DM). The importance of chemical constituents was evaluated with respect to the role of these plants in animal nutrition.

Keywords: *amino acids, fatty acids, minerals, plants, proximate composition.*

INTRODUCTION

In recent years, phytogenic feed additives have attracted increasing interest as an alternative strategy to replace growth promoters like antibiotics in farm animals (Cheng et al., 2014). In the European Union antibiotics have been banned completely from use as additives in livestock feed since 2006 because of a suspected risk of generating microbiota with increased resistance to the antibiotics used for therapy in humans and animals (Windisch et al., 2008).

Herbs have been used for medicinal purposes for centuries with a significant role in maintaining human health (Ertaset al. 2005). Plants and plant extracts are used in animal nutrition as appetite, digestion, physiological functions stimulants, as colorants and antioxidants or for prevention and treatment of certain pathological conditions. Keeping farmanimals healthy is necessary to obtain healthy animal products (Frankič et al., 2009). Phytogenic feed additives are plant-derived products used in animal feeding in order to improve performance of agricultural livestock and they have recently gained increasing interest, especially for use in monogastric animals (Windisch et al. 2008).

Fennel (*Foeniculum vulgare* Mil., *Apiaceae*) is a Mediterranean aromatic plant, which is used in traditional medicine and as a spice. Many studies have shown the diuretic, analgesic and antipyretic, anti-inflammatory, antimicrobial and antiviral, antimutagenic, antispasmodic, and antioxidant effect in the fennel fruit (Badgujar et al., 2014; Oktay et al., 2003). Kazemi Fard et al. (2013), reported thatthe addition of 50 mg/kg *Foeniculum vulgare* supplementation increased egg production, improved performance, hatchability and immune response in post molted broiler breeder hen.

The wild mint (*Mentha longifolia* L. family *Lamiaceae*) grows extensively in Mediterranean regions. *M. longifolia* is used in

the pharmaceutical and food industries. Different parts of the plant including its leaves, flower, stem, bark, and seeds have been also used for a long time in traditional folk medicine as antimicrobial, carminative, stimulant, antispasmodic and for the treatment of various diseases such as headaches and digestive disorders (Stamenkovic et al., 2005; Naghibi, et al., 2010). Durraniet *al*. (2007) reported that administration of *M. longifolia* to broiler chickens resulted in enhancement of weight gain, feed intake, water intake, feed conversion productivity, dressing percentage and weight of different body organs, and also significantly decreased mortality.

Salix alba L. it is known as the willow tree. The willow bark extract has a long history as a herbal remedy against fever, pain and inflammation, a given effect of salicylic acid (a precursor of aspirin) and the content in polyphenols and flavonoids (Anilkumar, 2010; März and Kemper, 2002; Nahrstedt et al., 2007).Saracila M. et al., 2018) found out that the dietary inclusion of willow bark extract (1%) in broilers diets did not show significant differences of the growth performance, but have shown a positive effect ($P<0.05$) compared with control, in reducing the proliferation of pathogenic bacteria (*Enterobacteriaceae, E.coli, staphylococci*) in the broiler caecum, under heat stress conditions.

The purpose of this study was to characterize some medicinal plants, from physico-chemical point of view and their potential to be used as a supplement in monogastric animal nutrition.

MATERIALS AND METHODS

Plant material

The studied plants used for the study were obtained from local pharmacies, dried, grounded and packed.

The choice of the plants investigated was based on the properties known and their use in the traditional medicine: mint *(Mentha longifolia)* leaves, fennel *(Foeniculum vulgare)* seeds and powder and white willow *(Salix alba)* bark extract.

Chemical analysis

Chemical methods were used to determine the concentration of the main nutrients from feeds according to Regulation (EC) no. 152/2009: gravimetric method for dry matter determination; Kjeldahl method for crude protein determination; the crude fat was determined by extraction in organic solvents; the crude fibre was determined by successive hydrolysis in alkali and acid environment and gravimetric method for crude ash determination.

The samples were analysed for Ca, Cu, Fe, Mn, Zn concentrations applying flame atomic absorption spectrometry (FAAS) after the microwave digestion. Each sample was processed as described previously (Untea et al., 2012) and a blank digest was carried out in the same way. Each sample was quantitatively transferred with 7 mL mixture of 65% HNO_3 : 30% H_2O_2 (5:2, v/v) into a 60 mL Teflon DAP – 60K vessels used fordigestion. Digestion conditions: 8 min at 130^0C, 80% energy; 5 min at 155^0C, 80% energy; 12 min at 170^0C, 80% energy. Maximal microwave oven power was 1000 W. After full cooling at room temperature the solutions were filtered through filter paper in a 50 mL volumetric flasks using boiling deionized water. The phosphorus content was determined by UV-Vis spectrophotometry according to Regulation (CE) nr. 152/2009.

In order to determine the amino acids profile of samples, an HPLC Surveyor Plus Thermo Electron (Massachusetts, United States), and HyperSil BDS C18 column (Thermo Electron, Massachusetts, United States), dimensions 250mm × 4.6 mm × 5 μm were used. The samples were prepared as described by (Varzaru et al., 2013).

Gas chromatograph Perkin-Elmer Clarus 500 (Perkin-Elmer, USA), fitted with Flame Ionization Detector (FID) and capillary separation column was used in order to determine the fatty acids composition of plant samples.

Each sample was prepared as described previously by (Panaite et al., 2016). The working principle is the saponification of the sample followed by extraction is petrol ether, concentration and addition of chloroform. The sample is split in the GC, it is separated in the chromatographic column, and the results are

compared with the standard chromatograms by measuring the peak area.

RESULTS AND DISCUSSIONS

Table 1 shows the concentration of the main nutrients (proximate composition) of the studied plants. The table shows that the highest level of crude protein is encountered in mint, followed closely by the fennel seeds.

The highest value of fiber it is found in fennel seeds (27.31%).

White willow bark had the highest level of dry matter (99.26%), and the lowest concentration in crude protein (0.18%).

The highest crude fat content was found in fennel powder (18.11%), while the lowest value was found in white willow bark extract (0.01%).

The highest value of ash content was 10.54% in mint and the lowest (0.46%) in white willow bark extract, fennel seeds and powder have similar levels (7.86% and 8.36%).

These results are in agreement with the literature on the protein content and crude fat in fennel (15.8 % and 14.87%), crude fat and fibre in mint (0.94% and 8.0%) reported by USDA, (2018).

Table 2 shows macro elements and trace elements concentrations in analysed samples. It can be seen that the highest concentration in Ca (1.50%), Fe (1408.98 ppm) and Mn (106.91 ppm) is encountered in mint.

Fennel seeds have important concentrations in Cu (14.97 ppm) and Zn (58.93 ppm), while fennel powder shows important concentrations in Fe (221.19 ppm) and Mn (52.88 ppm).

White willow bark extract does not have a rich content of macro and microelements. There were little variations of Ca, P, Cu, Zn content between fennel seeds, fennel powder and mint.

The Fe content shows great variations between the different plant species, from 3.41 ppm (willow) to 1408.98 ppm (mint).

Comparing the values obtained with the data presented in the literature, it can be observed no major differences on manganese and zinc (107.0 and 35.6 mg/kg) content in mint reported by Gogoasa et al., (2013).

Özcan and Akbulut, (2008) found the fennel content in Cu, Zn and Mn being 8.28 ppm, 20.8 ppm and 33.4 ppm respectively, the results being in the same range with our values.

Table 1. Proximate composition in analysed samples

Specification	Fennel seeds	Fennel powder	Mint leaves	White willow bark extract
Dry matter %	90.64	90.06	91.83	99.26
Crude protein %	18.89	17.18	20.72	0.18
Crude fat %	4.88	18.11	1.57	0.01
Crude fibre %	27.31	14.86	10.57	1.89
Crude ash %	7.86	8.36	10.54	0.46

Table 2. Macroelements and trace elements concentration in analysed samples

Specification	Fennel seeds	Fennel powder	Mint	White willow bark extract
Ca %	0.84	0.95	1.50	0.02
P %	0.53	0.49	0.29	0.39
Cu ppm	14.97	14.55	19.36	-
Fe ppm	37.85	221.19	1408.98	3.41
Mn ppm	27.11	52.88	106.91	0.41
Zn ppm	58.93	42.50	36.24	0.02

Table 3. Amino acids concentration in the analysed samples (%)

Amino acids	Fennel seeds	Fennel powder	Mint
Aspartic acid	2.614	2.543	2.702
Glutamic acid	4.170	4.189	3.136
Serine	1.296	1.186	1.271
Glicycine	1.387	1.401	1.346
Threonine	0.996	0.857	1.097
Arginine	1.235	1.126	1.226
Alanine	1.052	0.952	1.389
Tyrozine	0.157	0.115	0.215
Valine	1.239	1.099	1.652
Phenylalanine	1.104	0.920	1.494
Isoleucine	0.916	0.753	1.001
Leucine	1.483	1.231	2.081
Lysine	1.158	1.021	1.017
Cystine	0.298	0.254	0.179
Methionine	0.759	0.641	0.694
The total quantity of amino acids	19.865	18.289	20.498
Essential amino acids	7.655	6.522	9.036
Semi-essential amino acids	2.986	2.681	2.891

In Table 3 it can be seen that the highest values of essential amino acids like threonine, valine, phenylalanine, isoleucine and leucine are encountered in the mint. The content of lysine is higher in the fennel seeds (1.158%) than in the mint (1.017%) and fennel powder (1.021%). In the case of semi-essential amino acids, we noticed that, the glutamic acid is found in larger quantities in fennel powder and in fennel seeds than in mint. Cystine and methionine have higher concentrations in fennel seeds and mint have important alanine values.

As regards of total concentrations of essential amino acids, mint has the most important values semi-essential amino acids (9.036%), fennel seeds recording higher values than fennel powder. We can also see that fennel seeds have the highest value in semi-essential amino acids (2.986%) followed closely by mint (2.891%) and fennel powder (2.681%), values encountered in Table 4.

Table 4. Fatty acids concentration in the analysed samples (g/100 g fat)

Fatty acids		Fennel seeds	Fennel powder	Mint	White willow bark extract
Butiric	C 4:0	0.12	0.00	0.24	0.67
Caproic	C 6:0	0.47	0.10	1.91	6.72
Caprilic	C 8:0	1.20	0.13	8.32	0.24
Nonanoic	C 9:0	0.00	0.00	0.00	0.00
Capric	C 10:0	0.32	0.17	4.87	5.59
Undecanoic	C 11:0	0.03	0.39	0.22	0.03
Lauric	C 12:0	0.02	0.00	0.47	0.21
Tridecanoic	C 13:0	0.04	0.00	0.16	0.00
Myristic	C 14:0	0.71	0.82	8.51	10.36
Miristoleic	C 14:1	0.00	0.00	0.53	0.64
Pentadecanoic	C 15:0	0.21	0.08	0.82	1.38
Pentadecenoic	C 15:1	0.07	0.00	1.14	0.27
Palmitic	C 16:0	8.29	6.28	26.09	30.33
Palmitoleic	C 16:1	0.72	0.60	1.22	1.57
Heptadecanoic	C 17:0	0.11	0.00	0.41	0.69
Heptadecenoic	C 17:1	0.15	0.00	0.37	0.26
Stearic	C 18:0	1.96	1.42	7.11	9.37

Oleic cis	C 18:1	64.25	74.97	17.54	25.12
Linoleic cis	C 18:2n6	15.60	13.12	4.29	4.00
Arachic	C 20:0	0.02	0.00	0.03	0.27
Eicosenoic	C20 (1n9)	0.05	0.03	0.10	0.00
Linolenic α	C 18:3n3	1.19	0.44	11.12	0.75
Octadecatetraenoic	C18:4n3	0.58	0.27	0.55	0.29
Eicosadienoic	C20(2n6)	0.11	0.04	0.08	0.00
Behenic	C 22:0	0.33	0.18	0.19	0.00
Eicosatrienoic	C20(3n6)	0.09	0.08	0.06	0.00
Erucic	C22 (1n9)	0.65	0.37	0.00	0.00
Eicosatrienoic	C20(3n3)	0.67	0.06	0.10	0.00
Arachidonic	C20(4n6)	0.07	0.00	0.08	0.12
Docosadienoic	C22(2n6)	0.30	0.12	0.33	0.00
Tricosanoic	C 23:0	0.00	0.00	0.52	0.00
Eicosapentaenoic	C20(5n3)	0.13	0.00	0.07	0.00
Lignoceric	C24:0	0.13	0.00	0.07	0.00
Nervonic	C24 (1n9)	0.00	0.00	0.04	0.00
Docosatetraenoic	C22(4n6)	0.34	0.00	0.44	0.00
Other fatty acids		1.08	0.31	1.62	1.09
Total fatty acids		**100**	**100**	**100**	**100**
SFA		13.96	9.58	59.96	65.88
MUFA		65.88	75.98	21.30	27.86
PUFA		19.08	14.13	17.12	5.17
Ω3		2.57	0.77	11.84	1.04
Ω6		16.52	13.36	5.28	4.12
Ω6/Ω3		6.44	17.39	0.45	3.95

Similar results as regards isoleucine have been obtained by (Badgujar et al., 2014) in fennel (0.73%).

The fatty acids profile of analysed plants (Table 4) shows that the concentration of linoleic acid (C18:2n6) an omega 6 acid, was highest in the fennel seeds and fennel powder (15.60 g/100 g fat and 13.12 g/100 g fat) close values have been recorded for mint and white willow bark extract (4.29 g/100 g fat and 4.00 g/100 g fat). The concentration of linolenic acid (C18:3n3), omega 3 acid, was highest in mint (11.12 g/100 g fat).

The data of concentration of saturated fatty acids and polyunsaturated fatty acids (PUFA) has the greatest value in fennel seeds. Mint is good source of fatty acids with significant quantities of ω3 (11.84g/100g fat)and a ω3/ω6 ratio below 1 (0.45).

CONCLUSIONS

The mechanism for phytoadditives effects is not fully understood, but, based on chemical composition of plants, some of them can be considered to be used in animal nutrition. From the studied plants, it can be noticed that mentha is a very important source of trace minerals, essential amino acids and Ω3 fatty acids.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number **8PCCDI**/2018 Pc3, within PN-III-P1-1.2-PCCDI-2017.

REFERENCES

Anilkumar, M. (2010). Ethnomedicinal plants as anti-inflammatory and analgesic agents. *Ethnomedicine: A source of complementary therapeutics*, 267-293.

Badgujar, S.B., Patel, V.V., Bandivdekar, A.H. (2014). *Foeniculum vulgare* Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *BioMed research international*, 1-32.

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., Yuan, Z. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in microbiology*, *5*, 217.

Durrani, F., Sultan, A., Marri, M.L., Chand, N., Durrani, Z. (2007). Effect of Wild Mint (*Mentha longifolia*) Infusion on the Over All. *Pakistan Journal of Biological Sciences*, 10(7), 1130-1133.

Ertas, O.N., Guler, T., Çiftçi, M., DalkIIIç, B., Simsek, U.G. (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *International Journal of Poultry Science*, 4(11), 879-884.

Frankic, T., Voljč, M., Salobir, J., Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. *Acta Agric Slov*, *94*(94), 95-102.

Gogoasa, I., Violeta, J., Maria, A.L., Ariana, V., Maria, R., Alda, S., Gergen, I. (2013). Mineral content of some medicinal herbs. *Journal of Horticulture, Forestry and Biotechnology, 17*(4), 65-67.

Kazemi Fard, M., Kermanshahi, H., Rezaei, M., Golian, A. (2013). Effect of Different Levels of Fennel Extract and Vitamin D3 on Performance, Hatchability and Immunity in Post Molted Broiler Breeders. *Iranian Journal of Applied Animal Science*, *4*.

März, R.W., Kemper, F. (2002). Weidenrindenextrakt–Wirkungen und Wirksamkeit. Rkenntnisstand zu Pharmakologie, Toxikologie und Klinik. *Wiener Medizinische Wochenschrift*, 152(15,16), 354-359.

Naghibi, F., Mosaddegh, M., Mohammadi Motamed, M., Ghorbani, A. (2010). Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. *Iranian Journal of Pharmaceutical Research*, 63-79.

Nahrstedt, A., Schmidt, M., Jäggi, R., Metz, J., Khayyal, M.T. (2007). Willow bark extract: the contribution of polyphenols to the overall effect. *Wiener Medizinische Wochenschrift*, *157*(13-14), 348-351.

Oktay, M., Gülçin, İ., Küfrevioğlu, Ö.İ. (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT-Food Science and Technology*, *36*(2), 263-271.

Özcan, M.M., Akbulut, M. (2008). Estimation of minerals, nitrate and nitrite contents of medicinal and aromatic plants used as spices, condiments and herbal tea. *Food Chemistry*, *106*(2), 852-858.

Panaite, T., Criste, R.D., Ropota, M., Cornescu, G.M., Alexandrescu, D. C., Criste, V., Untea, A. (2016). Effect of layer diets enriched in Omega-3 fatty acids supplemented with Cu on the nutritive value of the eggs. *Romanian Biotechnological Letters*, 21(4), 11754-11762.

Regulations (EC) no 152/2009 laying down the methods of sampling and analysis for the official control of feed, Official Journal of the European Union L54. 2009.

Saracila, M., Tabuc, C., Panaite, T.D., Papuc, C.P., Olteanu, M., Criste, R.D. (2018). Effect of the dietary willow bark extract (*Salix alba*) on the caecal microbial population of broilers (14-28 days) reared at 32˚C. *Conference Proceedings "Agriculture for Life, Life for Agriculture"*, 1(1), 155-161.

Stamenkovic, V. (2005). *Our Non-Harming Medicinal Herbs*. Revised and Expanded. 2nd ed. Leskovac: NIGP Trend.

United States Department of Agriculture Agricultural Research Service National Nutrient Database for Standard Reference Legacy Releas, https://ndb.nal.usda.gov/, 2018

Untea, A.E., Criste, R.D., Vladescu, L. (2012). Development and validation of a liver samples preparation method for FAAS trace elements content determination. *Rev. Chim-Bucharest*, *63*, 341-346.

Varzaru, I., Untea, A.E., Martura, T., Olteanu, M., Panaite, T.D., Schitea, M., Van, I. (2013). Development and validation of an RP-HPLC method for methionine, cystine and lysine separation and determination in corn samples. *Rev. Chem*, 64, 673-679.

Windisch, W., Schedle, K., Plitzner, C., Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry, *Journal of animal science*, *86*(14_suppl), E140-E148.