Acta Sci. Pol., Technol. Aliment. 11(3) 2012, 303-310
pISSN 1644-0730 eISSN 1889-9594 www.food.actapol.net/

CHEMICAL COMPOSITION OF LUCERNE LEAF EXTRACT (EFL) AND ITS APPLICATIONS AS A PHYTOBIOTIC IN HUMAN NUTRITION

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ABSTRACT

Lucerne, a valuable plant grown mainly for animal feed, is rich in protein, minerals (Ca, Cu, Fe, Mg, Mn, P, Zn, Si), vitamins (A, B, C, D, E, K, U), phytochemical substances (carotene, chlorophyll, coumarins, isoflavones, alkaloids, saponins), contains secondary metabolites of plants (phytoestrogens: isoflavones and coumestrol), and antinutritional components (phytates, L-canavanine, saponins). It may be used as a dietary supplement in human nutrition. The proteins found in lucerne are comprised of numerous exogenous and semi-exogenous amino acids which are desirable for human body. Extract from the leaves of alfalfa (EFL – l'Extrait Foliaire de Luzerne) practically does not contain mycotoxins and pesticide pollutants. It is a completely natural product, safe even in a long-term supplementation. Extract from the leaves of alfalfa has a positive, multidirectional impact on the human body. It increases the level of estrogen, prevents atherosclerosis, helps blood circulation and strenghtens immunity, protects against the development of dangerous diseases of the digestive tract, combats anemia and many other health ailments. The results of preclinical studies indicate that alfalfa leaf extract enriched with vitamin C (EFL) can be a dietary supplement supporting the human body in fighting malnutrition, ischemic diseases, and various disorders of digestive tract. It also strengthens and enhances immunity.

Key words: lucerne, protein-xantophyll (PX) concentrate production, chemical composition, antinutritional and biologically active components, human dietary supplement EFL

INTRODUCTION

Lucerne is represented in our country by many species, but the major ones are *Medicago sativa* L. and hybrid lucerne (*Medicago* × *varia* Martyn T.). It was brought to Europe around 470 BC, during the so-called Medean wars. It was called Herba Medica (the herb of Media), which was later adopted as the name of the species *Medicago* [Zanin 2009]. The word alfalfa under which it is known in the Near East and in America means "the best forage". Currently, lucerne is one of the most commonly cultivated species in the world. The best conditions for its growth and development are in moderately warm or subtropical zones and in the uplands with the temperature of about 15-30°C with a balanced amount of rainfall and plentiful sunshine [Mauriès 1991, 1994]. This species tolerates short-term shortage of moisture in the soil.

Most often, lucerne is associated with animal feed. It is rarely used in human nutrition. Folk medicine uses this species because of its rich chemical composition and it is used it as an adjunct to pharmacological agents in the treatment of gastrointestinal, cardiovascular and immune system diseases [Głowniak et al.

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2007, Zanin 2009, Bertin et al. 2008]. This review was written with the aim to discuss the benefits to the human body of protein-xanthophyll (PX) concentrate also called lucerne leaf extract (EFL – Extrait Foliaire de Luzerne).

PREPARATION AND CHEMICAL COMPOSITION OF PX

In the middle of the last century in France, a method of obtaining a protein concentrate from the leaves of alfalfa was developed as a result of thermocoagulation of protein at the temperature of 85-90°C prior to conventional dehydration. Production of protein – xanthophyll extract (P-X) is carried out mainly in order to obtain a feed rich in protein, nutrients and vitamins for selected animal species [Zanin 2009]. The extract contains about 50% to 60% protein, macronutrients, vitamins, xanthophyll, and carotein. Its cellulose content is low, around 1-2% [Décision... 2009, Zanin 2009, Bertin 2008] (Tables 1 and 2).

Due to high content of protein and minerals, the composition of the PX concentrate from the leaves of lucerne is similar to the composition of milk powder and the best egg protein – spirulin [Zanin 2009, Bertin 2008] (Table 2). In the process of production of a protein – xanthophyll extract, drying of juice from lucerne takes place at a sufficiently low temperature so that the proteins associated with carotenoids and chlorophyll pigments retain their properties (pigments), and after its enrichment with large amounts of vitamin C (600 mg/kg), the product is stored in the refrigerator or in an inert gas without light exposure for use in human nutrition as lucerne leaf extract known as EFL from the French abbreviation of "l'Extrait Foliaire de Luzerne".

Table 1. Composition of the protein-xantophyll extract from alfalfa (*Medicago sativa*)

Protein	45-60%
Fat	9-11%
Simple sugars (soluble dietary fiber)	1-2%
Polisacharydes (insoluble dietary fiber)	11-15%
including cellulose	2-3%
Minerals	8-13%
Saponins	≤1.4%
Isoflavones	≤350 mg/kg
Coumestrol	≤100 mg/kg
Phytynians	≤200 mg/kg
L-canavanine	≤4.5 mg/kg

Source: Décision... [2009].

Table 2. Comparison of chemical composition of the extract from alfalfa leaves (EFL) with other protein products

Components, %	EFL	Whole powdered milk	Spirulin
Water	8	3	3-7
Total protein	55-58	26	55-56
Crude fat	9-10	26	4.7
Polyunsaturated fatty acids n-3	6.4 (4.8)	0.9 (0.2)	1.8 (1.0)
Minerals	13-14	8	7-9
Crude fiber	1-2	–	4-7

Source: Zanin [2009], Bertin [2008].

Chemical composition and value of lucerne biomass are affected by many factors including habitat conditions, weather, some agronomic treatments such as nitrogen fertilization, date of the first cut, cutting frequency [Gaweł and Żurek 2003], and genetic variability of lucerne varieties [Borowiecki and Gaweł 1998].

Green matter of alfalfa contains 17-22% of total protein, rich in non-essential (exogenous) amino acids, saturated fatty acids (palmitic, stearic), monounsaturated (oleic acid), polyunsaturated (linoleic, linolenic) fatty acids, vitamin A (β-carotene), B1, B2, B3 (PP), B5, B6, B8, B9, B12, C, D, E, K, U and minerals: Ca, Cu, Fe, Mg, Mn, P, K, Zn and Si, organic acids: malic and fumaric [Bertin 2008, Grela 2008, Grela and Kowalczuk-Vasilev 2010, Zanin 2009].

The crude fiber content in green matter of lucerne is relatively high (about 230.0-300.0 g/kg dry weight), and varies from variety to variety. Celluloses and

lignin fractions (ADF) constitute about 40-45% of dry weight [Gaweł and Żurek 2003]. However, protein – xantophyll extract EFL contains about 1-2% of crude fiber [Bertin 2008]. The rich chemical composition of the lucerne leaf concentrate makes it useful in human nutrition as a dietary supplement in the prevention of and combating various diseases. In animal nutrition, it is a phytobiotic which can replace antibiotic-based growth promoters [Grela 2008].

MAJOR ANTINUTRITIONAL AND BIOLOGICALLY ACTIVE COMPONENTS IN LUCERNE

The dry weight of lucerne includes not only the nutrients, minerals and vitamins, but also phytochemical substances: carotene, chlorophyll, coumarin, beta-sitosterol, fumaric acid, isoflavones, alkaloids, saponins, cryptoxanthin, daidzein, genistein, limonene, lutein, and zeaxanthin [Czech 2010, Goławska et al. 2010, Grela and Kowalczuk-Vasilev 2010, Semeniuk et al. 2008, Zagórka and Głowniak 2008, Stochmal et al. 2001]. Protein-xantophyll extract EFL, like the whole plants of alfalfa, contains secondary metabolites such as plant phytoestrogens: isoflavones and coumestrol and antinutritional components: phytates, L-canavanine and saponins.

Alfalfa varieties from different geographic regions contain different amounts of chemical anti-nutritive substances such as saponins. Most of them were found in the varieties of French origin, and the poorest in this component were varieties from Japan, Sweden and domestic varieties Radius and Tula [Borowiecki et al. 1999]. Saponins are glycosides which are soluble in water. They have foaming abilities and show soap-like action [Oleszek 2002]. In lucerne, saponins are present in higher amounts in roots than in aerial parts. The latter are used for animal feed where they account for 1.5% to 3% of total dry matter. The protein-xantophyll extract from alfalfa contains approximately 0.5-1.5% of saponins [Fenwick and Oakenfull 1983, Grela and Kowalczuk-Vasilev 2010]. In human body, saponins have an antiatherosclerotic effect, since they form insoluble complexes with cholesterol, which are then excreted in the feces [Głowniak et al. 2007]. Saponins also have an antibacterial, antifungal, and antiviral effect. They restrict the growth of protozoa, lower digestibility of protein, negatively affect human and animal reproduction, inhibit the growth of cancer cells. Saponins from the root parts show a stronger action than saponins from aerial parts of alfalfa [Francis et al. 2002, Szumacher-Strabel and Cieślak 2010, Szumacher-Strabel et al. 2010]. In addition to that, they show a strong antibacterial activity against gram-positive bacteria *Bacillus cereus*, *B. subtilis*, *Staphylcoccus aureus* and *Enterococcus faecalis*, and control some yeast-like fungi [Avato et al. 2006].

Phytoestrogens found in alfalfa such as biochanin A, daidzein, genistein belonging to isoflavones [Stochmal et al. 2001], as well as coumestrol enhance the level of oestrogens, so their action is similar to female hormones responsible for the development of the female reproductive tract [Zagórka and Głowniak 2008]. The use of the extract of aerial parts of alfalfa in studies on mice showed estrogenic effects of isoflavones and coumestrol on the increase of uterine weight in experimental animals [Stereva et al. 1977]. Phytoestrogens reduce symptoms of menopause in women, prevent osteoporosis and reduce the development of hormone-dependent breast and prostate cancers [Zagórka and Głowniak 2008, Rishi 2002]. Also elderly people can benefit from the application of plant isoflavones by improved memory and cognitive functions of the brain, increased bone density, as well as protection against breast, colon, and prostate cancer, as shown in an Indian study [Anklesaria 2011].

The toxic non-protein amino acid L-canavanine has been identified in larger quantities in the leaves (0.9-1.2 mg/g) than in the stems (0.6-0.9 mg/g), and the majority is present in the seeds and sprouts (80--150 mg/kg). However, it is much less abundant in the protein-xantophyll extract – PX (only 4.3 mg/kg) [Zagórka and Głowniak 2008] (Table 3). L-canavanine

Table 3. Content of L-canavanine in lucerne vs. other plants, µg/g of dry weight

Seeds of lucerne	Leaves of lucerne	Juice from lucerne	Protein--xantophyll extract from alfalfa (EFL)	Soy Flour	Lentil flour	Onions
80-150	10	110	4.3	2.1	2 800	10 000

Source: Bertin [2008].

has cytotoxic and antimetabollic properties. It disrupts the synthesis of DNA and RNA, and if consumed in large quantities, can cause autoimmune diseases in humans.

Another threat to humans are toxins of *Aspergillus flavus*, which are produced by mold fungi, and also pesticide residues and heavy metals in the feed. Bertin [2008] reported that protein-xantophyll extract from the juice of alfalfa (PX, EFL) contains a threshold quantity of harmful aflatoxins, ochratoxin, zearalenone and other mycotoxins and is free from pesticide residues. This is because chemical pesticides are not applied to lucerne grown for the EFL extract.

EFL IN HUMAN NUTRITION

Protein preparations of plant origin as human dietary supplements are permitted for use in the form of bakery products, beverages and sweets (chocolate, candy bars) and in capsules, tablets and in the form of powder in the U.S., Canada, Mexico, Peru. In 2009 in Europe, the European Commission allowed protein-xantophyll concentrate from alfalfa (EFL) to be used in human nutrition [Décision... 2009]. Rich and valuable chemical composition of EFL can be used to combat certain diseases in humans [Głowniak et al. 2007, Grela and Kowalczuk-Vasilev 2010, Zanin 2009], for example, it helps against the development of atherosclerosis as saponins form insoluble complexes of cholesterol and saponins (steroids) in the small intestine, which are then excreted from the body [Khaleel et al. 2005, Reshef et al. 2006, Zagórka and Głowniak 2008]. The high content of chlorophyll – an anticancer phytocomplex present in EFL has an impact on the gastrointestinal tract, and vitamin U accelerates healing of ulcers and relieves gastritis, shows antifungal activity, enhances the immune system, prevents anemia and detoxifies the body [Furgał and Milik 2008, Głowniak et al. 2007]. Phytoestrogens present in EFL act like soybeans and its products, and therefore alleviate symptoms associated with neurovegetative menopause in women, protect against hormone-dependent breast cancer, treat ischemic heart disease, and inhibit the process of osteoporosis [Anklesaria 2011, Rishi 2002, Seguin et al. 2004, Zagórka and Głowniak 2008]. Vyas et al. [2010] showed that the daily dose of EFL containing 5 mg of Fe and 13 µg of folic acid is just as efficacious and more cost-effective in the control of anemia than tablets containing 5 mg of Fe and 13 µg of folic acid. Preclinical studies conducted in India on a group of adolescent girls aged 14-18 years, patients with anemia, showed an approximately 15% increase in hemoglobin levels and an increase in the levels of other blood parameters as a result of administration of 10 g/day of alfalfa concentrate EFL [Bertin et al. 2008]. Similar results associated with increased amounts of hemoglobin and of other red blood cell indices were obtained by Taky et al. [2006]. In China, India and Romania, the concentrate from the leaves of alfalfa EFL used in the diets of older people in the amount of 15 g/day increased the content of hemoglobin, retinol, Ca and Mg in the blood. In young people, it improved mood and psychological state [Bertin et al. 2008]. It also improved exercise tolerance of young Polish athletes [Furgał and Milik 2008]. Research by Peiyou cited by Furgał and Milik [2008] comprised a group of 10 male students and 10 female students fed three times a day 2.5 g of EFL in addition to a portion of bread containing 37 g of cereal flour. The group was compared to an anologous control group (10 male students and 10 female students) which was fed bread only. The tested groups were tested for the level of hemoglobin. With a low initial hemoglobin level, 80 days of EFL intake resulted in a higher increase of hemoglobin content than that found in a non-EFL-treated control group (Table 4). Thus it was demonstrated that EFL prevented development of anemia by young women and men.

Observations of 50 individuals aged 50 to 86 years complaining of general weakness and malaise caused by low levels of hemoglobin, calcium, retinol and cholesterol who were fed daily doses of 15 g of EFL

Table 4. Hemoglobin level (g/dl) in the blood of students in China as affected by EFL intake

Group	Prior to test 1993.04.30	After test 1993.07.19	Increase
Tested – EFL 7.5 g/day	11.45	12.31	0.86
Control (no EFL)	11.45	11.97	0.52

Source: Milik (2008) after Peiyou [1993].

for 15 days showed an increase in hemoglobin by as much as 74% (in 37 persons), increases in the contents of retinol by 18% in the majority of the tested individuals, calcium by ca. 5% (68% of individuals) Mg by 38.5% (56% of individuals). The study also showed a substantial improvement in blood morphology in individuals who had tested positively for anemia [Furgał and Milik 2008 after Peiyou 1993].

A 12-year old swimmer suffering from acute idiopathic thrombocytopenia who had undergone both hospital and outpatient treatment including sterides was fed EFL together with vitamin C at doses increasing to 3 table spoonfuls a day [Furgał and Milik 2008]. Before the treatment, the boy had had the spleen removed and had been treated with immunodepresants. After 3 months of treatment with EFL there was an increase in the number of trombocytes and a decrease in the number of nuclear antibodies to their normal values. The young boy recovered and resumed swimming. Furgał and Milik [2008] described also another case of 2.5-year old child suffering from thrombocytopenia caused by iron deficiency. The child had undergone numerous unsuccessful hospital treatments with iron and sterides. The decision was taken to treat the child, along with the pharmacological treatment, with a daily dose of 5 g of EFL. After a month of that treatment red blood cell counts improved following the discontinuing of steride, immunoglobin and immunosuppresant treatment.

A study of 3 and 12 months' duration involving a group of malnourished children aged 3 to 5 years who showed a decreasing body weight/age index was conducted in 2004 in Peru and was described by Bertin [2010]. The children were feed EFL together with gruel made of 100 g of potato starch and 400 g of brown sugar in 2 l of water. Each child was fed 200 ml of gruel and 10 g of EFL with lemon juice. In the control group, the gruel was prepared in a different manner: 100 g of starch and 250 g of brown sugar were added to 2 l of water. In that group, the children were administered 250 ml of gruel and, additionally, 15 g of non-fat powdered milk (control group). Thus, in both groups – control and treated – equal contents of protein and carbohydrates were obtained. In order to demonstrate significant differences in the contents of hemoglobin, albumins, protein, and creatinine the t-Student test was used. In a three months' time, in the EFL group an improvement in blood hemoglobin and albumin contents was obtained and, in a year's time, the children in that group were faster in reaching normal levels of protein and albumins in the serum [Bertin 2010]. After a year's treatment with EFL Bertin [2010] did not find any serious side-effects even though such disorders as diorrhea, constipation and stomach pains did occur but they were transient and mild. The observations also showed that EFL is well tolerated by the human body and can be recommended to be used as a dietary supplement for as long as a year.

SUMMARY

The presented review of the literature shows that EFL is widely used in human nutrition and it exerts various positive effects on the human body. High nutritional value of EFL is associated with the favourable chemical composition of alfalfa, a high content of protein and amino acids, especially of valuable exogenous (non-essential) and semi-exogenous amino acids.

In human nutrition, extract from the leaves of lucerne enriched with vitamin C (EFL) can be used as a dietary supplement to increase the body's resistance and increases the amount of hemoglobin in blood. It can be used to enrich the proper microflora of the gut, relieving gastrointestinal disorders and anemia caused by lack of iron. It can also limit diarrhea, and improve mood. This preparation is suitable for long term use, because it does not show adverse effects on the human body. In addition to pharmacological treatment, it is useful to improve the health of people after chemo- and radiotherapy and of patients with HIV. Preclinical studies in humans suggest the possibility of using EFL to combat malnutrition, famine, and ischemic diseases of the digestive tract in the developing countries of the African continent, in South America and India.

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SKŁAD I ZASTOSOWANIE PREPARATU Z ROŚLIN LUCERNY EFL W ŻYWIENIU CZŁOWIEKA

STRESZCZENIE

Lucerna to wartościowa roślina paszowa, zasobna w białko, składniki mineralne (Ca, Cu, Fe, Mg, Mn, P, Zn, Si), witaminy (A, B, C, D, E, K, U) czy substancje fitochemiczne (karoten, chlorofil, kumaryny, izoflawony, alkaloidy, saponiny). Lucerna zawiera też wtórne metabolity roślinne (fitoestrogeny: izoflawony i kumestrol) oraz składniki antyodżywcze (fityniany, L-kanawanina, saponiny) i może być stosowana jako suplement diety w żywieniu człowieka. W skład białek występujących w lucernie wchodzą liczne aminokwasy egzogenne i semiegzogenne pożądane dla organizmu ludzkiego. Ekstrakt z roślin lucerny (EFL) praktycznie nie zawiera mikotoksyn i zanieczyszczeń pestycydami. Jest produktem całkowicie naturalnym, bezpiecznym nawet w długotrwałej suplementacji. Ekstrakt z roślin lucerny wykazuje wielokierunkowy, pozytywny wpływ na organizm ludzki. Działa estrogenie, przeciwmiażdżycowo, wspomaga układ krwionośny i odpornościowy, chroni przewód pokarmowy przed rozwojem groźnych chorób, zwalcza anemię i wiele innych dolegliwości zdrowotnych. Wyniki badań przedklinicznych wskazują, że ekstrakt z roślin lucerny, wzbogacony witaminą C (EFL), może być suplementem diety wspomagającym organizm człowieka w zwalczaniu

niedożywienia, chorób niedokrwiennych i różnych schorzeń przewodu pokarmowego, wzmacnia i zwiększa odporność organizmu.

Słowa kluczowe: lucerna, koncentrat białkowo-ksantofilowy (EFL), skład chemiczny, składniki antyodżywcze i biologicznie czynne, suplement diety

Received – Przyjęto: 21.09.2011 Accepted for print – Zaakceptowano do druku: 16.02.2012

For citation – Do cytowania

Gaweł E., 2012. Chemical composition of lucerne leaf extract (EFL) and its applications as a phytobiotic in human nutrition. Acta Sci. Pol., Technol. Aliment. 11(3), 303-310.