SOME BENEFICIAL EFFECTS OF LEGUME ANTINUTRITIVE SUBSTANCES

NEKI KORISNI UČINCI ANTINUTRITIVNIH SASTOJAKA MAHUNARKI

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SUMMARY

Legume plants are important source of feed for large population of animals in the world. They are characterised by the high level of valuable mineral elements but first of all constitute an important protein source. Different parts of legume plants are also consumed by people and they are especially important in the areas where the consumption of animal protein, for different reasons, limited. Beside the good value as a food or feed, legumes are also a rich source of different harmful (or even toxic) substances. It should be also taken into consideration that seeds are the most noxious part of plants.

The purpose of this presentation is to show that some potentially harmful substances included in legume plants can exert beneficial effects in animal nutrition.

Key words: legume plants, antinutritive substances, chemical composition, cultivars

CHEMICAL COMPOSITION OF LEGUME SEEDS

Legume seeds (Zduńczyk et al.,1994) are rich in protein and amino acids, especially in lysine (Table 1, 2), microelements (Table 3, 4), considerably lupin seeds are rich in manganese and dietary fibre, (Table 5, 6). The energetic value of legume seeds depends on both legume species and species of animals that fed on them (Table 7, 8, 9).

It is well known that legume plants and their seeds contain numerous, naturally occuring, antinutritional substances, which can be divided into many groups depending on their chemical properties, biological activity and potential harmfulness. Those include such non-protein substances as oligosaccharides; lectins and protease inhibitors, tannins, saponins, quinolizidine alkaloids, cyanogenic and pirymidine glycosides, phytates, isoflavones and some other that are less important and do not display a significant antinutritive activity.

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Table 1. Comparison of basal characteristics of legume seeds (%)
Tablica 1. Usporedba osnovnih značajki sjemenki mahunarki

Petterson and Mackintosh, 1994

Nutrient - Hranjive tvari	Faba beans Bob	Lupins Vučika	Field peas Ozimi grašak	Soybean meal Sojina sačma
Crude protein - Sirova bjelančevina	24.3	32.2-36.1	23.0	44.0
Total lysine - Ukupni lizin	1.5	1.5-1.52	1.56	2.8
Available lysine - Iskoristivi lizin				
- pigs - svinje	1.29	0.76-0.82	1.43	2.49
- poultry - perad	1.29	1.35-1.37	1.42	2.55
S-amino acids - S-aminokiseline	0.53	0.7-0.71	0.57	1.25
Tryptophan - Triptofan	0.16	0.41-0.42	0.21	0.52
Calcium - Kalcij	0.12	0.2-0.22	0.9	0.4
Phosphorus - Fosfor	0.44	0.3-0.36	0.39	0.60
ADF	7.2	14.3-19.7	8.5	11.0
Lignin	7.2	6.5-7.0	10.1	7.0
ME (MJ/kg)				
- cattle - goveda	13.4	11.7	10.9-12.0	13.7
- sheep - ovce	11.5	12.0	12.2-12.5	13.7
- poultry - perad	11.2	11.5	9.7-10.0	10.7

Table 2. Chemical composition of lupin cultivars harvested in 1991-1994, (% in DM)
Tablica 2. Kemijski sastav kultivara lupina branog 1991 – 1994 (% u suhoj tvari)

Wasilewko and Buraczewska,1999

Cultivars Kultivari	Crude protein Sirova bjelančevina	Ether extract Sirova mast	Ash Pepeo	Fibre fractions – Frakcije vlaknine			
				Crude fibre Sirova vlaknina	ADF	NDF	ADL
	Yellow lupin – Žuta vučika						
Juno	42.5	5.3	5.3	16.6	21.2	25.4	2.2
Popiel	44.1	5.3	5.1	15.2	20.0	24.1	1.8
Amulet	44.4	4.6	5.4	16.7	21.3	25.7	1.9
Radames	42.2	5.7	5.7	16.6	21.7	25.9	2.2
Cybis	44.1	5.6	5.4	15.8	19.7	24.6	1.5
Manru	43.6	5.5	5.3	17.3	21.7	26.8	2.8
	White lupin – Bijela vučika						
Hetman	37.4	10.8	4.6	13.0	17.9	21.6	2.5
Bardo	35.1	10.5	4.0	13.8	19.5	23.5	2.6
Wat	30.7	10.2	4.3	15.6	20.7	25.3	3.1
	Blue lupin – Plava vučika						
Emir	32.5	6.8	4.0	14.2	19.1	23.7	1.7
Sur	31.6	6.3	4.2	16.6	22.2	26.9	2.2
Saturn	34.6	5.9	4.3	15.8	21.2	25.2	2.0
Polonez	34.1	5.5	3.9	15.1	20.8	24.5	1.9

Table 3. Some microelements content in legume seeds (mg/kg DM)

Tablica 3. Sadržaj nekih mikroelemenata u sjemenkama mahunarki (mg/kg ST)

Matyka et al.,1997

Microelements Mikroelementi	Yellow lupin Žuta vučika	White lupin Bijela vučika	Blue lupin - Plava vučika		Faba beans Bob	Peas Grašak	Field peas Poljski grašak
			Sweet Slatka	Bitter Gorka			
Fe	81	43	64	60	63	73	86
Zn	80	53	41	44	65	49	56
Mn	**125!**	**292!**	110	101	18	16	20
Cu	11.2	6.2	5.1	5.1	12.2	7.0	7.9
Co	0.54	0.47	0.46	0.40	0.60	0.37	0.44
Se	0.37	0.18	0.23	0.23	0.16	0.13	0.16

Table 4. Mineral composition of lupin cultivars harvested in 1991-1994

Tablica 4. Mineralni sastav kultivara lupine branih 1991 - 1994

Wasilewko and Buraczewska,1999

Cultivars	Ca	K	P	Mg	Na	Fe	Mn	Zn	Cu
Kultivari	g/kg DM				mg/kg DM				
	Yellow lupin - Žuta vučika								
Juno	2.4	14.0	8.9	3.1	89	90	50	78	8.7
Popiel	2.8	12.6	7.9	2.8	103	93	102	86	11.8
Amulet	3.2	12.4	7.7	3.6	98	85	37	74	10.0
Radames	3.8	13.3	8.8	3.0	99	80	70	82	10.5
Cybis	3.4	12.0	7.6	3.6	111	128	48	76	10.0
Manru	4.3	12.3	7.6	2.6	79	99	85	72	8.9
Yellow lupin – Žuta vučika	3.3	12.8	8.1	3.1	96.4	95.7	65.2	77.9	10.0
	White lupin - Bijela vučika								
Hetman	3.9	11.8	5.0	2.1	96	63	828	46	5.7
Bardo	3.5	11.5	4.4	1.7	126	53	742	47	5.7
Wat	3.3	12.4	5.0	2.0	134	62	447	50	6.7
White lupin – Bijela vučika	3.6	11.9	4.8	1.9	118.7	59.2	672.3	47.8	6.1
	Blue lupin – Plava vučika								
Emir	3.1	12.3	5.2	1.9	97	84	54	43	5.8
Sur	3.5	10.8	4.8	2.1	90	63	58	43	5.7
Saturn	4.3	10.3	4.6	2.8	112	61	26	37	3.4
Polonez	2.9	10.6	5.1	2.0	79	39	29	33	3.5
Blue lupin – Plava vučika	3.4	11.0	4.9	2.0	94.5	61.7	41.8	39.1	4.6

Table 5. Chemical composition of whole and dehulled seeds of faba beans (% in DM)
Tablica 5. Kemijski sastav čitavih i oljuštenih sjemenki boba (% u ST)

Frejnagel et al.,1997

Chemical composition - Kemijski sastav	Nadwiślański	Tinos	Caspar
	Whole seeds – Cijele sjemenke		
Ash - Pepeo	3.2	3.4	3.3
Crude protein - Sirova bjelančevina	30.0	29.0	24.2
Ether extract - Sirova mast	0.9	0.8	1.2
Dietary fibre - Hranidbena vlaknina	22.8	22.0	21.0
	Dehulled seeds – Oljuštene sjemenka		
Ash - Pepeo	3.36	3.7	3.4
Crude protein - Sirova bjelančevina	33.2	31.6	26.2
Ether extract - Sirova mast	1.0	1.0	1.4
Dietary fibre - Hranidbena vlaknina	14.5	13.5	12.6
	Hulls - Ljuske		
Ash - Pepeo	2.8	3.0	2.9
Crude protein - Sirova bjelančevina	5.3	5.4	5.7
Ether extract - Sirova mast	0.1	0.1	0.1
Dietary fibre - Hranidbena vlaknina	76.4	73.1	80.6

Table 6. Chemical composition of the peas cultivars (g/kg)
Tablica 6. Kemijski sastav kultivara graška (g/kg)

Igbasan et al.,1997

Cultivar Kultivar	Crude protein Sirova bjelančevina	Dietary fibre Hranidbena vlaknina	Starch- Škrob	Crude fat- Sirova mast	Ca	P	Tannin
Express	207.5	210.8	436.6	17.9	0.6	2.9	<1.0
Highlight	214.8	209.9	412.0	14.8	0.9	4.2	
Baroness	225.7	203.2	433.9	18.2	0.9	5.3	
Titan	225.9	200.2	436.8	18.5	0.6	5.1	
Fluo	229.4	202.0	415.3	21.3	0.8	5.1	
Montana	243.3	196.3	397.8	13.4	0.8	4.4	
Impala	245.2	192.3	400.8	14.3	0.8	3.6	
Tara	264.0	190.7	385.3	14.5	1.3	4.3	
Radley	232.2	192.5	425.1	20.1	0.7	3.2	
Trump	244.9	202.8	424.6	20.2	0.7	4.4	
Carman	255.0	214.3	392.8	14.7	0.9	5.6	11.5
Sirius	231.9	223.1	415.3	12.4	0.8	3.0	41.0
Mean[1]	235.0	203.2	414.7	16.7	0.8	4.3	5.2
SD[2]	15.53	9.44	16.77	2.88	0.18	0.88	11.17

Tannin content was based on catechin equivalents
[1] srednja vrijednost
[2] standardna devijacija

Table 7. Energetic value in some legume seeds (MJ/kg DM)
Tablica 7. Energetska vrijednost nekih sjemenki mahunarki (MJ ME/kg ST)

Brinkmann and Abel,1996

Species	MJ ME/kg DM MJ ME/kg ST (suhe tvari)
Horse bean - Bob	9.2-12.8
Peas - Grašak	9.5-13.1
Lupins - Vučike	
- White lupin - bijela	6.8-10.7
- Yellow lupin - žuta	8.2-9.6
- L. mutabilis	12.4

Table 8. Energetic value in four varieties of faba beans
Tablica 8. Energetska vrijednost u četiri vrste graha (MJ ME/kg ST)

Abel and Römer,1996

Color of flowers	Varieties	Broiler-Brojleri		Swine-Svinje
		ME	$ME_{N\text{-korr}}$	ME
White	Albatros	13.6 ±0.6	11.5 ±0.5	15.6 ±0.3
	Caspar	12.6 ±0.7	11.5 ±0.7	14.5 ±0.4
Colored	Alfred	11.7 ±0.7	10.6 ±0.6	13.2 ±0.3
	Scirocco	9.9 ±1.3	7.8 ±1.2	12.4 ±0.3

Table 9. Energetic value of the legume seeds
Tablica 9. Energetska vrijednost sjemenki mahunarki

Matyka et al.,1997

Item	Poultry-Perad		Swine-Svinje	
	kcal/kg DM	MJ/kg DM	kcal/kg DM	MJ/kg DM
Yellow lupin	2285	9.56	3556	14.04
White lupin	2091	8.75	3277	13.71
Blue lupin	1960	8.20	3219	13.47
Faba beans	2727	11.41	3451	14.44
Peas	3033	12.69	3755	15.71

Main antinutritive effective substances in legume seeds:

- Oligosaccharides (alpha-galactosides)
- Lectins
- Inhibitors of proteases and alpha-amylase
- Tannins
- Saponins
- Phytoestrogens
- Phytates
- Antigenic proteins
- Alkaloids

Oligosaccharides

The physiological role of structural substances (dietary fibre) in the gastrointestinal tract of animals is relatively well-known. The physical properties of fibre fractions such as water-holding capacity and solubility, high viscosity, capacity to cation exchange and ability to bind different minerals and blocking bile acids plays an important role in the digestion process that can affects the metabolic pathways. Fibre fractions are useful in prevention and/or in easing of constipation, enhance the intestine motility, and to a great extent influence the passage of digesta through the intestine.

Selected carbohydrates that stimulate the immune response against the antigens presence show different activities:

Carbohydrates	Substantial function in fat synthesis; biosynthesis of triacylglycerols and cholesterol
Oligosaccharides (prebiotics)	Derivatives of fructose and galactose; **Support the colon functions, show anti-cancer activity**; undigested and unabsorbed – decrease the energy supply to the intestine; **decrease insulin secretion**; **products of microbiological fermentation of oligosaccharides (SCFA) in colon can exert profitable effect on the mucosa cells proliferation in the intestine wall**; show the **anti-inflammatory activity and improve the intestine motility;**
NSP – β-1,3/1,6-glucans	Adhese to the sugars - receptors of leukocytes (monocytes, macrophages, granulocytes)

Indigestible substances – especially flatulence induced oligosaccharides (α-galactosides: e.g. raffinose, stachyose, verbascose) – occur mainly in seeds containing great amounts of protein (rape seeds, legume seeds – table 10,11,12,13). These saccharides are not digested in the intestine of monogastric animals due to the absence of endogenous enzymes (*α-galactosidase*) in the intestinal mucosa (Trevino et al.1995). This enzyme is necessary to break the α1→6 linkages. From other sources as well as from practice it is well known that the intestinal digestion of α-galactosides can be improved by supplementation of animal diets with exogenous microbial α-galactosidase. Escape from digestion these saccharides are stored in the distal part of the large intestine (colon) where they can negatively affect the processes of digestion and absorption of nutrients (Smiricky et al., 2002). High concentration of α-galactosides in colon of monogastric animals may have a beneficial effect. They favor the intensive anaerobical bacterial fermentation (can promote the growth of *Bifidobacteria*) and e create considerable quantities of the short-chain fatty acids that are the source of

Table 10. The indigestible oligosaccharides content in poultry feed (g/DM)
Tablica 10. Sadržaj neprobavljivih oligosaharida u hrani za perad

(Mosenthin, 2001)

	Soybean	Soybean meal	Lupin	Peas	Faba beans	Wheat	Barley	Maize corn
Alpha-oligosaccharides	29-59	42-73	101-123	30-113	-	5	2	3
Stachyose	30-52	47	42-55	23	16	1-2	0-1	1
Raffinose	7-19	10	10-11	5	4	4-5	2-5	2
Verbascose	-	3	23-40	22	34	-	-	-
Sucrose	-	70	14-39	30	27	8-11	12-14	13
Fructo-oligosaccharides	-	-	-	1	-	1-8	2-7	-

energy for intestinal mucosa, but also CO_2 and methane (CH_4) which is considered to affect negatively animal production. Accumulation of α-galactosides may be useful in keeping the colon healthy and in decreasing cancer incidence in this intestinal part. In young ruminants the α-galactosides activity is manifested by the loss of appetite (even anorexia), excretion of small quantities of dense faeces, lack of sounds of digestion and by the presence of hard, dense, detectable particles of feed inside rumen.

Table 11. The oligosaccharides content in different legume seeds
Tablica 11. Sadržaj oligosaharida u sjemenkama raznih mahunarki

(Piotrowicz-Cieślak,2005)

Species	Raffinose	Stachyose	Verbascose
	g/kg DM		
Cicer arietinum	4.5	17.2	1.0
Glycine max	12.6	43.4	trace
Lupinus albus	8.2	41.1	4.8
Phaseolus limensis	6.9	30.3	trace
Phaseolus lunatus	4.6	27.6	3.1
Phaseolus vulgaris	2.6	21.6	0.3
Phaseolus vulgaris	4.3	26.2	trace
Phaseolus aureaus	13.9	16.7	26.6
Pisum sativum	6.0	17.1	23.0
Pisum sativum	11.6	32.3	19.1
Vicia faba minor	2.2	6.7	14.5
Vicia faba bean	2.3	10.7	11.4

Table 12. Non-starch polysaccharides profile (% of total saccharides) of the pea cultivars
Tablica 12. Profil neškrobnih saharida (% ukupnih saharida) kultivara graška

(Igbasan et al.,1997)

Cultivar	Rhamnose	Arabinose	Xilose	Mannose	Galactose	Glucose	Uronic acid
Express	0.4	18.4	5.5	0.5	3.4	48.5	23.3
Highlight	0.4	20.6	6.1	0.5	3.9	46.9	21.5
Baroness	0.6	17.8	5.2	0.4	4.7	48.2	23.0
Titan	0.5	18.5	6.9	0.5	3.4	47.1	23.1
Fluo	0.4	21.5	7.1	0.6	4.6	41.1	24.7
Montana	0.4	21.4	7.0	0.5	4.6	44.4	21.7
Impala	0.5	19.1	6.0	0.5	4.0	47.1	22.9
Tara	0.4	19.8	7.1	0.6	3.7	48.5	19.9
Radley	0.4	17.2	5.8	0.4	3.8	51.0	21.4
Trump	0.5	18.8	6.9	0.5	3.7	49.5	20.2
Carman	0.4	19.0	6.8	0.5	4.5	44.7	24.0
Sirius	0.4	18.3	5.5	0.6	4.3	51.7	19.2
Mean	0.4	19.2	6.3	0.5	4.1	47.4	22.1
SD	0.06	1.31	0.68	0.06	0.45	2.82	1.64

Table 13. Carbohydrate composition of three varieties of faba beans hulls (g/kg DM)
Tablica 13. Sastav ugljikohidrata triju vrsta boba

(Longstaff and McNab,1991)

Variety	Medes	Brunette	Minica
Rhamnose	7.8	5.1	5.3
Fucose	2.4	1.6	1.8
Arabinose	15.5	12.9	11.0
Xylose	143.7	80.8	86.6
Mannose	2.2	2.0	2.0
Galactose	21.3	18.3	18.7
Glucose	597.0	542.6	561.6
Uronic acid	171.2	138.4	139.6
Total	961.2	801.7	826.6

Lectins (hemagglutynins)

The role of lectins in legume plants is controversial (Grant and Driessche,1993., Berić et al.,1997). These glycoproteins usually occur in small quantities in legume seeds - Soya beans, beans, but do not occur in lupins (Table 14, 15, 16). They are also present in some mushrooms and in live organisms e.g. in physiological liquids of lower *Chordata*, fish' eggs (roe) and in cellular membranes in mammals.

Table 14. Specific chemical composition of different faba beans (g/kg)
Tablica 14. Specifičan kemijski sastav raznih vrsta boba

(Abel and Römer,1996)

Content	Colored flowers	White flowers
Hemagglutynin activity	27.10	27.20
Total phenols	20.10	4.50
Tannins	14.10	0.14
Condensed tannins	26.20	nd
Trypsin inhibitor activity	1.85	3.05
Saponins	3.17	1.83
Phytates	16.60	5.00

Table 15. Antinutritional factors content (in DM) and nutritional value of field bean protein
Tablica 15. Sadržaj antinutritivnih tvari (u ST) i hranidbena vrijednost bjelančevina poljskog graha

(Pastuszewska et al.,1993)

Field bean Poljski grah	Lectins (mg/g)	Tannins (%)	Trypsin inhibitor (mg/g)	True digest. %	Biological value %	Net protein utilization
Toret	3.20	0.10	1.93	89.8c	83.8a	75.3b
Caspar	nd	0.10	1.75	85.9b	85.6abc	73.5b
Pistache	0.40	0.29	2.26	83.6b	84.7ab	70.8ab
Kamir	2.90	0.42	2.16	83.0b	89.6bc	74.4b
Martin	2.30	0.55	1.74	78.3a	90.5c	71.0ab
Alfred	2.70	0.55	1.39	78.6a	84.3a	66.3a

Differences marked a, b, c are significant at $P<0.05$

Table 16. Anti-nutritional factors in sweet lupins
Tablica 16. Antinutritivne tvari u slatkoj lupini

(Petterson and Mackintosh,1994)

Factors	Units	Range	Mean
Alkaloids	%	0.005-0.043	0.019
Oligosaccharides	%	4.73-6.14	5.16
Phytate	%	0.37-0.91	0.58
Total tannins	%	0.17-1.20	0.32
Condensed tannins	%	<0.01—0.01	<0.01
Saponins	mg/kg	442-740	573
Trypsin inhibitor activity	mg/g	<0.01-0.28	0.14
Chymotrypsin inhibitor activity	mg/g	<0.01-0.59	0.08
Lectins	dilution	Not detected	Not detected

The lectins from various sources differ from each other in the degree of antinutritional activity. They are the main toxic substances in the raw beans (*Phaseolus vulgaris* L.), which is one of the major human foods in some areas.It is well known that some lectins found in legume seeds can cause serious health problems in both humans and animals. Many of them, but not all, are capable to agglutinate red blood cells followed by hemolysis and even death of an animal. Also in vitro lectins show the red blood cells agglutinating properties as a result of binding of sugars or glucoproteides (Liener,1989., Pusztai,1989., Koninkx et al.,1993 a,b). The toxicity of lectins depends on their capacity to adhese to the specific sugar receptors on mucous layer of intestine. Lectins given *per os* can affect the intestinal mucosa and induce acute enteritis, diarrhea, bloating and some other disturbances in the gastrointestinal tract. So, in effect, they can inhibit the growth and development of animals. In acute, long-lasting incidences lectins can cause even death of animals. Applied parenterally, lectins can affected the immune response and decrease the resistance against infections (Bardocz et al.,1999., Czerwiński, 2004).

Another important nutritional function of lectins is the capacity to inhibit the absorption of nutrients from intestine because of the adhesion to the cells' receptors of intestinal epithelium. Accumulation of huge amounts of nutrients in the intestine and inhibition of absorption of B-group' vitamins can increase the pool of substrates that become available to the not always profitable intestinal microflora and in this way can promote their proliferation. In humans fed diets containing high amounts of beans (*Phaseolus* spp.) the diarrhea, nausea, bloating and vomiting as an effect of unprofitable microflora proliferation were observed (Muzquiz et al.,1999).

The physiological role of lectin in animals has not been fully recognized yet. Their noxious activity can concern different tissues. It has been established that they can induce the occurrence of necrotic focuses in liver; in stomach and intestine local bleedings can occur.

Molecules of lectins can be blocked by simple monosaccharides, however substances that are inhibited by di-, tri- and polysaccharides also exist. Lectins are deactivated during the steam treatment. This procedure can inhibit the harmful activity completely. If the share of legume seeds in the diet does not exceed 10 % it is possible to fully eliminate the noxiousness of these compounds (Peumans et al.,1996). Some lectins present in various plants, including legumes, are obviously toxic, but usually they are given in small quantities only together with the legume feed. It could be supposed, that the dietary lectins are harmless because the daily intake of their active form is too low to exert noxious effects and the risk of acute toxicity is low.

Lectins are weakly degradable in the intestine and are transported in a reactive form through the digestive tract. They:

- **have strong properties to adhese to the epithelial cells**,
- influence the cellular metabolism,
- complementary bind carbohydrates (glucose/mannose) on the external surface of intestine which affects the cell wall metabolism,
- **are the growth factor for the small intestine weight,**
- lead to hypertrophy and hyperplasia of Lieberkühn crypts and cell size enlargement (up to 25 %),
- induce crypt' cells division,
- **increase the pancreas and liver growth,**
- **strongly interfere with pancreas insulin secretion,**
- reduce the thymus size,
- interact with endocrine cells on the gut epithelium affecting release of gastrointestinal hormones,
- some lectins (kidney beans) induce catabolism of adipose tissue lipids,
- inhibit growth.

A research exists which indicates that the legume lectins are capable of inhibiting the *Escherichia coli* proliferation in the rat's intestine (Biernat,2002., Kruszewska et al., 2003., Czerwiński, 2004). Some plants (e.g. from *Allium* family) in their edible parts contain similar lectins as legume plants and have some prebiotical activities. So, it has been suggested that similar effects could be expected from legume lectins.

The proteases inhibitors

In the raw feed components, apart from the compounds blocking the activity of the vitamin-like substances, the presence of other compounds blocking the activity of enzymes important for there correct course of metabolic processes was also detected. Those compounds constitute a large and diverse group of substances characterized by the antinutritive activity. The proteinous inhibitors of enzymes are relatively well recognized; the highest activity of these substances was found in soya seeds and their negative effects depend mainly on the plant' genotype. From the nutritional viewpoint the source of proteases inhibitors are the cereal grains, mainly rye and triticale, legume seeds - horse bean, peas, lupins, soybeans (Table 17, 18, 19, 20) as well as several oily seeds, such as sunflower (Birk, 1961). Among these substances both Kunitz and Bowmann-Birk trypsin inhibitors, present in soybeans are the best recognized ones because of their importance in human alimentation (Janicki et al., 1970). The antinutritive activity of these proteins in relation to the endogenous enzymes of the gastrointestinal tract in both humans and animals depends on the presence of the sulphuric bonds in the rest of amino acids. Its unfavorable activity depends on blocking the proteolytic enzymes secreted in digestive tract by pancreas (the trypsin and chymotrypsin) and results in the hypertrophy of this organ. These substances in effect reduce the digestibility and absorption of dietary proteins.

Table 17. Influence of three varieties of faba bean hull polysaccharides and tannins on enzyme activity in the digesta
Tablica 17. Utjecaj polisaharida i tanina triju vrsta boba na aktivnost enzima u digestu

(Longstaff and McNab,1991)

Varieties	Tannin content (% catechin equivalent)	Trypsin (EC 3.4.21.4) (µg N-aniline/g digesta)	α-Amylase (EC 3.2.1.1) (mg maltose/g digesta)	Lipase (EC 3.1.1.3) (Sigma-Tietz units/g digesta)
Control	-	1708a	66.8a	73.8a
Medes	0	1412a	75.4a	61.1b
Brunette	1.50	464c	25.3c	37.9c
Minica	2.10	313c	27.3c	36.8c

Faba bean share in the diets = 400 g/kg
Differences marked a, b, c are significant at P<0.05

Table 18. Some antinutritive substances in legume seeds (g/kg DM)
Tablica 18. Neke antinutritivne tvari u sjemenkama mahunarki

(Matyka et al.,1997)

Antinutritive substances	Lupinus luteus	Lupinus albus	Lupinus angustifolius		Vicia faba	Pisum sativum	Pisum arvense
			Sweet	Bitter			
Trypsin inhibitor (TIU/mg CP)	0.15	0.60	0.0	-	8.2	13.1	11.0
Alkaloids	1.47	1.84	3.11	25.4	-	-	-
Phytates	16.7	6.8	83	-	13.5	9.6	10.5
Tannins	7.4	9.0	9.1	-	14.1	6.2	10.2

Table 19. Antinutritional factors in whole and dehulled faba bean seeds (% DM)
Tablica 19. Antinutritivni čimbenici u čitavim i oljuštenim sjemenkama boba (% ST)

(Frejnagel et al.,1997)

Antinutritive factors	Whole seeds			Dehulled seeds		
	Nadwiślański	Tinos	Caspar	Nadwiślański	Tinos	Caspar
Phenolic compounds						
Total phenols[1]	1.35	1.69	0.27	0.27	0.14	0.25
Proanthocyanidine[2]	0.85	1.11	0.02	0.02	0.02	0.01
Flavanols[3]	0.29	0.38	0.04	0.04	0.03	0.02
Trypsin inhibitor activity, TUI/mg	6.32	7.39	3.45	4.30	3.89	4.00
Glycosides						
Vicine	2.28	5.78	4.11	nd	nd	nd
Convicine	1.39	3.38	3.43	nd	nd	nd
Total	4.67	9.16	7.54	nd	nd	nd
Inositol phosphates	1.01	1.06	0.94	1.14	1.19	1.04
α-galactosides						
Stachyose	0.90	0.77	0.94	nd	nd	nd
Raffinose	0.10	0.08	0.13	nd	nd	nd
Verbascose	1.36	1.20	1.18	nd	nd	nd
Total	2.36	2.05	2.25	nd	nd	nd

1 – sinapic acid equivalent;
2 – cyanidine chloride equivalent
3 – D-catechin equivalent

Table 20. Inhibition of enzymes by various bean and pea seed-coat water extracts (PVP)
Tablica 20. Inhibiranje enzima od vodene otopine ljuske različitih vrsta graha i graška

(Griffiths,1981)

Varieties	Inhibition (%)					
	Alpha-amylase		Trypsin		Cellulose	
	Control	+PVP	Control	+PVP	Control	+PVP
Beans						
Triple White	4.1	3.3	6.5	6.4	1.8	2.0
Minden	64.9	0.1	51.4	8.3	12.8	7.7
Peas						
Filby	5.4	4.6	7.2	8.0	1.8	1.7
Minerva	54.5	1.4	65.8	8.9	9.9	2.8

In legume seeds the inhibitors of enzymes content is relatively high. So, it can negatively affect the nutrients absorption in animals fed diets containing raw, untreated seeds. The trypsin inhibitors found in other plants, such as potatoes, can also negatively affect the digestibility and biological value of the protein from diet.

The opinions on the stability of antinutritive activity of protease inhibitors are varied. The trypsin inhibitors content can be reduced or limited by the use of suitable techniques of feeds preparation or some other substances such as tannins (Pisulewska and Pisulewski, 2000., Jansman et al., 1994). These substances could be inactivated by the high temperature applied during preparation (Table 21).

Table 21. Effect of processing of faba beans on the level of trypsin inhibitor, hemagglutinin (lectins) and vicine, and degree of gelanization of starch

Tablica 21. Učinak prerade boba na razinu inhibitora tripsina, hemaglutinina (lektina) i vicina te stupanj želatiniranja škroba.

(Marquardt et al.,1976)

Treatment of faba bean	Trypsin inhibitor activity mg/g	Hemagglutynin activity Units	Vicine OD 660 nm	Gelatinization mg maltose/g
Control	1.8	2700	0.11	106
Microwave (20 min)	0.3	1300	0.11	98
Microwave (30 min)	0.2	1300	0.12	100
Steam pelleted (70 °C)	1.5	2700	0.11	108
Extruded (130 °C)	0.4	300	0.12	180
Extruded (152 °C)	0.2	200	0.13	250
Autoclaved (121 °C)	0.2	40	0.11	108

Both trypsin and chymotrypsin inhibitor activity do not depend on the total protein level in the diet. In peas, about 10 % of the total trypsin inhibitors is found in hulls and 90 % in cotyledons and these amounts in peas are highly correlated.

The structure of these substances is closely related to the structure of Bowmann Birk inhibitor and to the structure of Kunitz' trypsin inhibitor and they **can show the anti-cancer activity.** They are stable in temperature below 80 °C, weakly inactive in temperature about 100 °C and are totally degraded during extrusion (Marquardt et al., 1975).

Tannins

These substances are widely present in the plants and the most abundant amounts of them are found in legume seeds such as peas, faba beans (horse bean), vicia spp., also in sorghum, sunflower, rape seeds etc. (Sell et al., 1985., Jansman,1993) (Table 22). They constitute an element of the defense-line of plants against the harmful live elements of their environment (Robbins et al., 1987). In plants and seeds they occur mainly in the thin coat of the surface of the fruits, leaves, stalks, stems etc. (Table 23). Tannins do not participate in metabolic processes in plant tissues, however after degradation of plant cells they can reach the metabolic pathways in animals (Table 24).

Table 22. The tannin content in legume seeds
Tablica 22. Sadržaj tanina u sjemenkama mahunarki

(Jansman and Longstaff,1993)

Legume seeds	Tannin content (g/kg)	Reference
Chickpeas (*Cicer arietinum*)	0.8-2.7*	Rao a. Deosthale,1982
Faba bean (*Vicia faba* L.)	0.8-7.0	Griffiths,1981
	0.5-13.1**	Bos and Jetten,1988
	9.5-24.0**	Wang and Ueberschar,1990
Peas (*Pisum sativum* L.)	5.0-10.5***	Reddy et al.,1985
	0.6-3.5**	Griffiths,1981
	0.0-7.0**	Stickland,1984
Dry beans	0.3-2.8*	Deshpande et al.,1982
(*Phaseolus vulgaris* L.)	0.3-3.3*	Deshpande and Cheryan,1985
	2.3-12.6*	Salunkhe et al.,1990
Cereal grains		
Sorghum (*Sorghum vulgare* L.)	0.5-72.0*	Salunkhe et al., 1990
Barley (*Hordeum sativum* L.)	5.5-12.3***	Eggum and Christensen,1975

* Catechin equivalents; ** tannic acid equivalents; *** Folin Denis assay

Table 23. Distribution of polyphenols and condensed tannins in testa and cotyledons of the bean and pea varieties (% in air-dried material)
Tablica 23. Raspodjela polifenola i kondenziranih tanina u testumu i kotiledonima vrsta graha i graška

(Griffiths,1981)

Variety	Polyphenols(tannic acid equivalents)			Condensed tannins(catechin equivalents)		
	Testa	Cotyledons	Whole seeds	Testa	Cotyledons	Whole seeds
	Beans					
Maxime	7.70	0.91	1.61	4.20	0.08	0.59
Minden	7.70	0.91	1.92	4.20	0.08	0.70
Triple White	0.28	0.84	0.70	0.04	0.07	0.06
	Peas					
Marathon	5.25	0.46	0.85	2.86	0.08	0.23
Minerva	6.65	0.50	1.05	3.75	0.10	0.35
Filby	0.28	0.56	0.50	0.03	0.08	0.06

Table 24. Correlations between some dietary parameters and indices of faba beans evaluation
Tablica 24. Korelacija između nekih hranidbenih pokazatelja procjene boba

(Pastuszewska et al.,1993)

X	Y	Correlation coefficient
Tannin content in faba bean	Protein digestibility (TD)	-0.94*
Tanin content in faba bean	Net protein utilisation (NPU)	-0.69
Tannin intake	Feed/gain ratio (FCE)	-0.08
PER	NPU	0.95*

* significant P<0.01

In aqueous dilutions they are able to create the complexes with proteins, enzymes and other great-molecular substances such as carbohydrates, minerals (incl. metals) and vitamins. They can also adhese to the membranes of bacterial cells. Tannins differ among themselves because of their molecule weight that can vary between 500 to 3000 Da and are divided into two groups: condensed polymers and easy hydrolysed compounds. The latter are not a problem in animal feeding, but condensed tannins are polyphenolic substances that are very common in plants and are considered to be antinutritional compounds. The level of tannins in peas and faba beans depends on the varieties (color of flowers) and fractions of legume seeds (Table 23).

Tannins can interact with proteins in feed (protect them against too fast degradation e.g. in the rumen), with proteins in microbial cells, proteins of saliva, endogenous proteins as well as with other feed constituents (McMahon et al., 2000., Marquardt and Ward,1979). This activity can to a great extent reduce the protein digestibility in monogastric animals. They can decrease feed intake, lower the protein utilization thus decreasing the growth rate. In monogastric animals large amounts of tannins can negatively affect the digestive proceses mainly through inhibition of trypsin activity and limitation of the nutrients absorption from the intestine (Jansman et al.,1993., Nyachoti et al.,1996). **In ruminants it is possible to observe the slightly positive effect especially in cases of inhibition of bloat development** (Sweeney et al., 2001., Makkar, 2003). Tannins level exceeding 40-50 g kg^{-1} in the diet can reduce the digestibility of both protein and dry matter in ration given to ruminants but **low levels of condensed tannins slow down the protein degradation and increase the quantity of dietary protein, especially essential amino acids, flowing to the small intestine in ruminants and improve the protein utilization** (McMahon et al., 2000).

The high doses of tannic acid can limit or reduce intestinal absorption of some compounds -glucose, methionine, vitamin B_{12}. This can result from creating the insoluble complexes in the intestine or from strong influence of the tannic acid on the intestinal epithelium.

Hardly accessible are the information related to the activity of tannins in frugivorous birds and in fish, however it was established that application of tannins to a fish diet (carp *Cyprinus carpio* L.) limits the oxygen use in fish. It was also proved that tannic acid is more toxic to fish as compared to other compounds of tannin complex (Becker and Makkar, 1999).

In people as well as in animals the saliva rich in proline is secreted. This amino acid can assimilate tannins and in this way protect the feed proteins against their binding in complexes with tannins. However, the long-lasting consumption of tannin-rich diets can cause the enlargements of salivary glands. **By inhibiting the growth of bacteria** that cause tooth decay tannins can be useful in keeping hygiene of the mouth. **The tannin-protein complexes (e.g. tanalbine) are applied in human and animal medicine as a prophylactic substance and styptic agent and antidiarrhoeal drug. Tannins were also attributed to have a slight, positive role in limitation of parasitic invasions and reduction of the pathogens activity.**

The tannins given in feed through a long period of time can inhibit the enzymatic (proteins) activity, they can also be absorbed to the blood as well as induce damages to capillary vessels and liver (steatosis, necrotic focuses etc.). **The physiological activity of the tannins also depends on limiting the permeability of intestine walls**. This phenomenon has the unquestionable, unfavorable influence on the degree of feed utilization, but on the other hand the limitation of **permeability of intestine walls can be successfully used in cases of intake of some toxic substances by animal. Because of their ability to bind the proteins tannins can be used as a factor in removing some toxins from the intestine.** These complexes (tannin-toxin), however, are unstable, so, should be removed from the intestine very fast because of the danger of secondary degradation and absorption.

Saponins

Saponins are diverse, glycosidic compounds foaming aqueous solutions. These compounds are created from one or more sugar molecules and aglycons – that usually have triterpene or steroid structure. The presence of polar and non-polar groups in the structure provide saponins with strong surface-active properties that determine their adverse biological activity. In relatively small quantities they are present in many plants and have been identified in legume seeds - beans, peas, soya, lupin, but also in green and dried clover and lucerne (Table 14,16), sunflower seeds as well as in some sea animals. Generally, they are recognized as toxic.

Saponins, as high molecules, are absorbed from the intestine very slowly, however, the absorbed quantities enter very fast different metabolic pathways, where they are converted in to the cyclic compounds (steroids) often possessing a large healing properties. **Some saponins are applied in human medicine as antitussive drugs.**

Majority of saponins are bitter and can reduce appetite and the feed intake and can negatively affect the growth rate, especially in monogastric animals. The growth inhibition has bean observed in chickens, the smaller utilization of the feed compounds as well as worsening of the absorption of nutrients and vitamins. In ruminants a strong link between both the decrease of rumen motility and frequency of bloat due to saponins rich feeds (clover, lucerne, soya) has been found. It was also stated that saponins can inhibit *Protozoa* proliferation and are able to change the rumen fermentation (Hu et al., 2005).

Saponins are able to decrease the surface tension of liquids creating less or more foamed solutions. It is especially important in the cases of bloat in ruminants. They can easily penetrate the lipidous layer of the cell wall. There is an opinion that the function of saponins depends on increasing the permeability of intestinal walls, which can be important for better utilization of vitamins and mineral salts (mainly Ca and Fe) (Milgate and Roberts, 1995). However, this opinion has not yet been entirely verified.

Beneficial activity of saponins depends on their activity in lowering the cholesterol level. Perhaps it is also possible to limit the heart disease incidence. The anticarcinogenic activity in humans is possible (they can inhibit the colon cancer development). Some saponins possess bacteriostatic and antifungal properties. Triterpenic saponins can irritate the stomach mucosa and act as the expectorants, they can increase the secretion of stomach' juice and bile acids, intensify the absorption of nutrients from the intestine, emulsify fat and positively affect the lipids digestion. Other symptoms of the influence of the saponin are the stimulation of the sweat and salivary glands activity of as well as probably the secretory action of kidney and liver.

On the other hand some saponins can create insoluble complexes with Fe, Zn and Ca reducing their absorption and possibly cause deficits of these elements. Southon et al. (1988) fed rats on diets containing saponins obtained from the roots of *Gypsophila* (the family *Caryophyllaceae*) and observed the clear impairment of the iron management in those animals.

Some glicosidic saponins (e.g. digitogenin) absorbed into blood act as strong protoplasmic poisons strongly hemolizing the erythrocytes. Toxic activity of these compounds concerns first of all the parenchymatous organs. As the result of damage to capillary vessels there the interstitial bleedings in kidneys and in liver, damage of alveoli as well as haematomas in walls of small intestine can occur. Some saponic glycosides can cause the acute inflammation of the mucosa of alimentary tract accompanied by vomiting and diarrhoea; some other can negatively affect the central nervous system causing convulsions and paralyses.

Phytoestrogens

Phytoestrogens constitute a group of about 20 estrogen-like substances found in plants and in feeds of herbivorous animals. Estrogens in both humans and animals are the hormones that are necessary for reproduction. It was also proved that these hormones are strictly connected with bone and heart health in women. On the other hand, high estrogen secretion during a woman's lifetime is clearly linked with increased risk of breast cancer. Phytoestrogens are not stored in the organism and can be easily degraded and then eliminated from the body.

The main sub-groups of dietary phytoestrogens occurring in legume plants (Table 19) are isoflavones, lignans and coumestans. Isoflavonoid phytoestrogens (isoflavones) are found primarily in raw soya and in soya products (one of the richest sources). Lignan phytoestrogens (e.g. enterolactone) are found mainly in oilseeds (flaxseed *Linum sativum* L.) but also in foods/feeds containing high levels of fibre, such as cereal brains or beans. The coumestans can be detected in many different legume plants, such as lucerne or clover.

Two main phytoestrogens present in soybean (*Glycine max*) – daidzein and genistein (also glycitein, equol and other) - act as antagonists to the naturally occurring hormone - estradiol, inhibiting its activity. This phenomenon can exert a beneficial effect in adults, but can be deletorious in young animals (calves, piglets). Both mentioned substances are estrogenic, but have weaker effects than estrogens. The estrogenic properties of phytoestrogens can induce different disturbances of the reproduction processes. In sheep the permanent infertility and in geese the disturbances in reproduction have been observed. Among other the genistein has been shown to have both estrogenic and anti-estrogenic activity depending on the dose applied in the diet. Stevenson et al. (2006) state that genistein can act as an estrogen in the chickens oviduct and that dietary exposures to genistein may alter oviduct development.

It is supposed that these compounds, like estrogens, in humans do not induce the proliferation of cells and block specific receptors making the cells "safe" against more active forms of sex hormones. They prevent the cells from proliferation and possible mutations and are capable of inducing the *apoptosis* (cell death) of cancer cells. However this phenomenon has been found in vitro with breast cancer cells only (Sethell, 2001., Messina,1999).

Flavonoids are absorbed from the gastrointestinal system by resorption and by bacterial metabolism in the rumen and intestine. Inside organisms they inhibit the oxydation-reduction system (redox system) through the arrest of activiy of some enzymes such as cholinesterase, xanthic oxydase, estrogenic synthetase and others. Some of them show **antibacterial, antiviral and anti-inflammatory activity** and can **inhibit the free radicals** and **liver toxins. They can act also as an antiallergic and detoxifying factor.** These compounds are coenzymes **participating in carbohydrate metabolism of heart muscle and increase the resistance of vacuole' walls**, as well as **induce activity of enzymes oxidising some cancerogenic compounds**. They also **inhibit oxydation of the LDL cholesterol fraction and can act as diuretics.**

Phytic acid (phytates)

Phytic acid (myo-inositol 1,2,3,4,5,6 hexakis-dihydrogen phosphate) is a normal constituent occurring in legume seeds (Tab. 14,16,18). The antinutritional effects of phytic acid are connected primarily with six very reactive phosphate groups (Figure 1) that are responsible for the poor essential minerals bioavailability. It binds trace- and macro-elements such as Zn, Ca, Mg and Fe inside gastrointestinal tract. Formed complexes are indigestible, so, dietary minerals are unavailable for absorption and further utilization. This acid can also bind proteins and some enzymes in the intestine (Singh and Krikorian,1982).

Plenty of phosphorus in cereals and other feedstuffs occur in phytic acid salts – phytates, which are unavailable to monogastric animals or are hydrolised to a very small extent only.

The unfavorable effect of phytates on the bioavailability of minerals could be, however, **used in a profitable way. These compounds lower the solubility of calcium, fluoride and phosphate** that are the main constituents of tooth enamel. In this way the teeth **are more protected against the action of acids and/or bacteria – the main causes of teeth caries.**

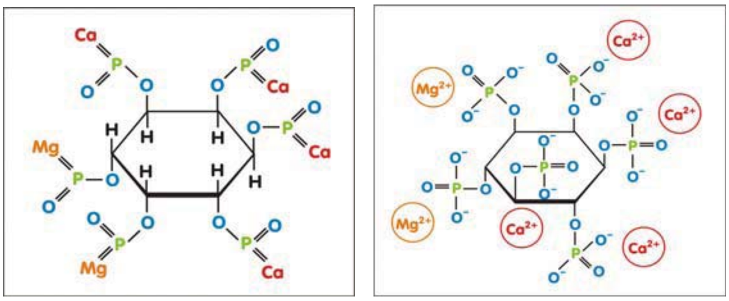

Figure 1. The structure of phytates (Source: Internet)
Slika 1. Struktura fitaza (Izvor: Internet)

High fibre-rich feeds, and in them also high amounts of phytates, can be useful in prevention of organism against cancer, especially in large intestine.

Antigenic proteins

Soybeans contain antigenic proteins which act allergenically in young animals (calves, piglets) and influence negatively morphology of the gastrointestinal wall – causing shortening of villi height, reduction of T-lymphocytes in the intestine epithelium (Table 25).

Table 25. Comparative data obtained in calves and piglets fed diets containing antigenic soyabean flour on the morphology and cellular immunology of the proximal jejunum

Tablica 25. Usporedni podaci dobiveni za telad i prasad hranjenu obrocima s antigenskim brašnom soje o morfološkoj i celularnoj imunologiji proksimalnogjejunuma

(Lallés et al.,1996)

Item	Preruminant calves		Piglets
	% SMP	% HSPI	% HSPC
	T lymphocytes in the lamina propria		
CD2+	324	172	146
CD4+	128	75	212
CD8+	179	109	101
WC1+	435	118	12
	B lymphocytes in the lamina propria		
Ig+ (total)	205	89	391
IgM+	102	0	178
IgA+	254	170	168
IgG1+	294	124	506
IgG2+	167	78	507
	Morphology		
Villus height	-43	-14	-27
Crypt depth	+1	-5	0
Villus height/crypt depth	-36	-10	-27
	T lymphocytes in the epithelium		
CD2+	80	10	145
CD4+	300	191	533
CD8+	110	121	30
WC1+	64	59	92

SMP – skim milk powder; HSPI – hypoantigenic soya bean protein isolate; HSPC – hypoantigenic soybean concentrate

Quinolizidine alkaloids

Lupins have many beneficial properties. Because of their role in nitrogen fixation, they are very important ecological factors. Lupin seeds are rich in proteins, which make them interesting for use in animal nutrition, but contain also several secondary metabolites used for chemical defence. Among them the most important are the alkaloids. Amountsand chemical properties of those vary significantly in different lupin species (Table 26). One of the most often found (over 70) lupin alkaloids are the substances of quinolizidine group (Pistelli et al, 2001).

Table 26. Comparison of alkaloids content in seeds of Polish forage and bitter lupine varieties (g/kg DM)
Tablica 26. Usporedba sadržaja alkaloida u sjemenkama "Polish forage" i vrsta gorke lupine

(Matyka et al.,1997)

Lupine variety	Range	Mean
	Yellow lupin	
Afus	1.76-3.62	2.45
Topaz	0.73-2.58	1.13
Wentus	0.53-1.32	0.83
Orbit	0.97-1.26	1.10
Pałucki	0.74-1.17	0.98
Aga	1.86-2.23	2.04
Bas	1.16-1.28	1.22
Mazowiecki	1.07-1.25	1.16
Sam	1.08-1.10	1.09
	Blue lupin	
Ignis	2.83-8.33	5.25
Emir	0.64-1.07	0.84
Kazan	0.73-1.45	1.19
	White lupin	
Wat	1.65-2.20	1.84
	Blue lupin bitter	
Mirella	20.28-25.16	22.91
Turkus	25.61-30.34	27.14
Wielkopolski	23.61-30.75	26.20

Most European lupin varieties are characterized by a relatively high concentration of alkaloids. The examples of these can be lupinine, lupanine and sparteine and others (Table 27).

Quinolizidine alkaloids are derivatives of lysine and have the double quinolizidine ring in their structure and are anti-nutritive when present in higher concentrations. They occur mainly in bitter lupin seeds, which results in small usefulness of these seeds as feed for animals. The richest in toxic substances is the yellow lupin, then white and as the least toxic the narrow-leaved (blue) lupin. The toxicological effect of quinolizidine alkaloids depends on their activity mainly in relation to the nervous system. In rats the adverse effects of fertility and other reproductive indices have not been observed. In dogs and cats the harmful effect of sparteine and lupanine on some neurological functions have been indicated.

Table 27. Some quinolizidine alkaloids in major lupin species
Tablica 27. Neki alkaloidi kvinolizidina u glavnim vrstama lupina.

(adapted from Wink et al,1995)

Alkaloids	Lupin species			
	albus	*angustifolius*	*luteus*	*mutabilis*
Albine	15	-	-	-
Lupanine	70	70	-	46
Multiflorine	3	-	-	-
13-hydroxylupanine	81	12	-	12
Angustifoline	-	10	-	1
Lupinine	-	-	60	-
Sparteine	-	-	30	16
Ammodendrine	-	-	-	2
Tetrahydrohomfifonine	-	-	-	2
3-hydroxylupanine	-	-	-	12

Some of them might be interesting as health promoting food additives. Some researches indicate **that lupinine and gramine have a bacterioicidal effect on some bacteria genus** in concentrations of less than 10 mM. **Lupanine, angustifoline, sparteine and some other could exert a inhibitory effect on *E. coli, Pseudomonas aeruginosa, Bacillus thuringiensis, Bacillus subtilis* and *Staphylococcus aureus*.** Sparteine in concentrations of 15 mM shows an antimicrobial effect on pathogenic fungi, such as *Altenaria porri, Piriculata oryzae, Helmintosporium carbonum, Fusarium oxysporium* and *Aspergillus oryzae*) (cit. Tyski et al., 1988., Wink, 1984 – taken from Jul et al. 2003).

Other antinutritional substances

Other antinutritional substances (glycosides and others) are present in peas (Table 28) and vicia spp. but the number of investigations on their antinutritive activity is relatively small. Low quantity e.g. vicia seeds are applied in the diets for pet-birds, for poultry this kind of legume seeds is subtoxic.

Vicine, convicine - pyrimidine glycosides that occur in faba beans undergo the hydrolysis in the digestive tract by a bacterial β-glucosidase aglycones and isouramil → absorbed into blood, where the free radicals lead to a reduction of glutathione pool in erythrocytes → haemolytic anemia → favism; These substances also increase the spleen weight, decrease the liver weight, blood glucose concentration, lymphocyte number as well as egg size, fertility and hatchability.

Table 28. Amount of major storage proteins, legumin and vicilin in seeds from two pairs of near-isogenic peas, determined by immunoelectrophoresis
Tablica 28. Količina glavnih zaliha bjelančevina, legumina i vicilina u sjemenkama dva para skoro-izogeničkog graška, određena imunoelektroforezom

(Hedemann et al.,1999)

	LA5	HA5	LB5	HB5
Legumin (g/kg DM)	47.3	55.8	53.6	53.6
Vicilin (g/kg DM)	56.3	57.0	55.7	64.7
Legumin (g/kg protein)	240.6	289.8	260.5	262.4
Vicilin (g/kg protein)	289.4	326.5	270.2	315.5

Some practical results of investigations with monogastric animals

Use of pea seeds in the diets of broiler chickens reduced the body weight and feed conversion but had no negative influence on the carcass quality, SCFA production in the caeca and on the endogenous enzymes activity (Table 29).

Significant increase of α- and β-galactosidases activity was observed in rats fed mixtures with lupins and pea seeds (Table 30).

Application of peas (Longstaff and McNab,1987) and faba beans or other legume seeds in animal' diets decreased both the starch and lipids digestibility (Table 31). Thermally processed legume seeds (faba beans) had a significant profitable effects on feed conversion, body weight gain and weight of inner organs (Table 32).

This method and duration of preparation (extrusion, autoclaving) reduced the activity of hemagglutynin and trypsin inhibitor (Table 33, 34).

Legume species and extrusion significantly influenced the microbial status of the small intestine content (Table 35), weight of particular parts of the gastrointestinal tract (Table 36, 37), height of villus and depth of crypts of the small intestine (Table 38).

Without effect on the haematological parameters in broiler chickens was e.g. the gramine content (from lupin), although significant differences induced by high or low gramine concentration in the diet were noted in the uric acid concentration in serum and in the amino acid content in plasma (Table 39, 40).

Table 29. Influence of pea and enzymes supplements on ducks performance
Tablica 29. Utjecaj graška i enzima na proizvodne rezultate pataka

(Jamroz et al., 2000)

	Exp	Kind of mixture			Enzyme supplement	
		Control	Peas (%)		-	100 ppm
			15/17	25/30		
Body weight (g)						
49 d	I	2453a	2491ab	2578b	2457A	2567B
	II	2636A	2462B	2515B	2505A	2569B
Feed conversion (kg/kg BW)						
0-49 d	I	1.982a	1.995a	2.058a	2.049a	1.983a
	II	1.934A	2.029B	2.122C	2.047a	2.09b
Dressing percentage (%)	I	70.4a	71.6ab	71.7c	71.0a	71.4a
	II	72.9a	71.1ab	69.9c	71.1a	71.5a
SCFA in caeca content (mmol/kg)	I	213.7	221.4	189.0	202.0	214.0
	II	113.9a	153.1b	155.0b	125.7a	155.6b
DM of caeca content (%)	I	20.4	21.5	21.1	21.2	20.8
	II	22.5	21.6	22.9	22.5	22.1
Trypsin activity (U/g pancreas tissue)	I	0.68a	0.54b	0.67a	0.61	0.65
	II	0.99	1.34	1,36	1.32	1.15
Alpha-amylase activity	I	18.4	17.8	21.3	19.0	19.4
(U/mg pancreas protein)	II	19.0A	14.1a	7.9b	14.1a	13.2a
Lipase activity (U/mg pancreas protein)	I	4.85	5.27	5.21	4.87	5.35
	II	5.70	6.19	6.09	6.44	5.55

Differences marked with A, B differs significantly at P<0.01; Differences marked with a, b differs significantly at P<0.05

Table 30. Body weight gain and caecal parameters in rats fed experimental diets
Tablica 30. Prirast tjelesne mase i pokazatelji cekuma štakora hranjenih pokusnim obrocima
(Juśkiewicz et al.,2003)

Indices	Oligosaccharides of		
	Control	Lupins	Peas
Body weight gain, g/14 days	54.6	60.8	60.9
Diet intake, g/14 days	245	249	246
Caecal tissue, g/100 g BW	0.33Bc	0.41Aa	0.38Ab
DM of caecal digesta, %	22.6A	19.5B	17.8C
pH of caecal digesta	7.03Bb	7.15ABb	7.37Aa
Ammonia concentr. in caecal digesta, mg/g	0.24B	0.51A	0.46A
Lowry's protein in caecal digesta, mg/g	0.13C	0.24A	0.19B
Total SCFA, umol/100 g BW	112.5AB	98.5B	129.1A
Acetate	66.2Ab	54.0Bc	76.1Aa
Propionate	21.9b	21.9b	28.8a
Isobutyrate	2.6Bb	3.3Aa	3.2ABa
Butyrate	15.3	13.5	12.6
Isovalerate	2.6b	2.6b	4.0a
Valerate	3.6AB	3.0B	4.3A
$C_2 : C_3 : C_4$ profile, µmol/100 µmol total SCFA	59:20:14	55:22:14	59:22:10
Enzyme activity (U/g fresh caecal content)			
β-glucuronidase	0.49Bb	1.89Aa	0.93ABb
β-glucosidase	0.25	0.28	0.32
β-galactosidase	1.68Bb	14.14Aa	8ABb
α-galactosidase	0.60Bb	6.86Aa	3ABb
α-glucosidase	1.06B	3.21A	2.90A
Enzyme activity (U/g protein)			
β-glucuronidase	76b	152a	96ab
β-glucosidase	38a	22b	32ab
β-galactosidase	260Bb	1142Aa	872ABb
α-galactosidase	93Bb	553Aa	370ABb
α-glucosidase	164Bb	261ABa	295Aa

Total α-galactosides (% DM) extracted from *Lupinus angustifolius* – 48.5; from *Pisum sativum* – 61.0
Differences marked with A, B differs significantly at P<0.01; Differences marked with a, b differs significantly at P<0.05

Table 31. Influence of three varieties of faba bean (*Vicia faba* L.) on the digestibility of starch and lipid
Tablica 31. Učinak tri vrste boba (*Vicia faba* L.) na probavljivost škroba i masti

(Longstaff and McNab,1991)

Faba bean varieties	Cofficients of digestibility (%)	
	Starch	Lipids
Control	97.7A	87.4A
Medes	91.2B	80.9B
Brunette	74.8A	76.4
Minica	53.8B	65.5

The share of faba beans in mixtures = 400 g/kg

A, B – differ significantly at P<0.01

Table 32. Effect of processing of faba beans on inner organs in broiler chickens
Tablica 32. Učinak prerade boba na unutarnje organe tovnih pilića

(Marquardt et al.,1976)

Treatment of faba beans	Weight gain (kg)	Feed:gain ratio (g/g)	Weight (g/kg)		
			Liver	Pancreas	Spleen
Control	541c	2.01a	27	3.2a	1.4a
Microvawe (20 min)	535c	2.09a	26	2.7bc	1.4a
Microvawe (30 min)	571bc	1.99a	26	2.6c	1.4a
Steam pelleted (70 °C)	572bc	1.85b	25	3.0bc	1.5a
Extruded (130 °C)	602ab	1.66c	24	2.8bc	1.4a
Extruded (152 °C)	626a	1.62c	25	2.6c	1.5a
Autoclaved (121 °C 20 min)	632a	1.80b	26	2.4c	1.5a
SEM	11	0.03	1	0.1	0.1

Differences marked with a, b differs significantly at P<0.05

Table 33. The influence of legume species or treatment on protein concentration and trypsin activity in pancreas of rats
Tablica 33. Učinak vrste mahunarki ili tretmana na bjelančevine i aktivnost tripsina u gušterači štakora

(mean values; Leontowicz et al.,2001)

Item	Pancreas % BW	Protein mg/pancreas	Trypsin activity U/total pancreas	Trypsin activity U/100 g pancreas	Digestibility (%)	
					Dry matter	Crude protein
Influence of seeds (n=10)						
Faba bean	0.316a	12.6a	0.343a	2723a	93.3a	84.7ab
Peas	0.306a	10.4a	0.327a	3162a	93.2a	86.1b
Soybean	0.361b	12.4a	0.376a	3097a	92.1b	84.0a
Influence of treatment (n=15)						
Raw	0.346b	13.0b	0.361a	2777a	92.6a	83.9a
Extruded	0.309a	10.6a	0.336a	3212b	93.1b	86.0b

Differences marked with a, b differs significantly at P<0.05

Table 34. Effect of autoclaving time of faba beans on the level of certain components in faba beans and on the performance of birds

Tablica 34. Učinak vremena autoklaviranja boba na razinu nekih komponenata u bobu i performance ptica

(Marquardt et al.,1976)

	Autoclaving time (min)					
	0	3	10	20	30	40
Faba bean component						
Gelatinization (mg maltose/g)	130	120	120	120	120	120
Vicine (OD 650 nm)	0.11	0.11	0.11	0.11	0.11	0.11
Hemagglutynin activity (units)	2.690	2.100	1.350	81	0	0
Trypsin inhibitor (units)	1.00	0.40	0.11	0.10	0.04	0.04
Bird performance						
Initial weight (g)	91	91	91	91	91	91
Gain (g)	330b	321b	333b	364ab	385a	383a
Feed : gain ratio (g/g)	3.18a	3.26a	3.14a	2.84b	2.66b	2.66b
Liver weight (g/kg)	25	26	25	25	25	25
Pancreas weight (g/kg)	4.0a	3.7a	3.2b	3.2b	2.9b	2.9b

Differences marked with a, b differs significantly at P<0.05

Table 35. Microbial parameters of small intestine in rats fed diets with 10 % soybean or faba bean (n=10)

Tablica 35. Mikrobielni pokazatelji u tankom crijevu štakora hranjenih s 10 % soje ili boba (n=10)

(Leontowicz et al.,2001)

Diets with legume seeds	Total culturable bacteria, log CFU/g	E. colilog CFU/g	E. coli/total culturable bacteria counts
Faba bean, raw	5.81 <0.93b	4.62 <1.03b	0.795
Extruded	5.32 <0.86ab	3.59 <1.11a	0.675
Soya bean, raw	4.81 <0.80a	3.00 <0.44a	0.624
Extruded	4.85 <1.12a	2.78 <0.97a	0.573

Differences marked with a, b differs significantly at P<0.05

CFU – colony forming units

Table 36. Gastrointestinal segments and organ relative weights (%BW) in rats fed diets with 10 % faba bean, pea or soybean (n=20)

Tablica 36. Gastrointestinalni segmenti i relativne mase organa (%BW) u štakora hranjenih obrocima s 10% boba, graška ili soje (n=20)

(Leontowicz et al.,2001)

	Faba bean	Pea	Soybean
Stomach	0.551b	0.500	0.537
Small intestine	2.561b	2.271	2.501
Caecum	0.396b	0.330	0.352
Colon + rectum	0.479ab	0.437	0.514
Large intestine*	0.875b	0.767	0.866
Kidney	0.732c	0.656	0.688
Heart	0.363ab	0.353	0.393
Liver	4.384a	4.424	4.350
Spleen	0.223b	0.188	0.194

Differences marked with a, b differs significantly at P<0.05

* Large intestine – caecum + colon + rectum

Table 37. Organ relative weights (g/100 g BW) in rats fed diets containing legume globulin (G) proteins

Tablica 37. Relativne mase organa (g/100 g BW) u štakora hranjenih obrocima koji su sadržavali bjelančevine mahunarki /globulin (G)

(Rubio et al.,1999)

	Lacto- albumin	Faba bean G	Lupin G	Soya G	Pooled SD
Stomach	0.62a	0.76b	0.75b	0.73b	0.05
Spleen	0.35a	0.24b	0.25b	0.29ab	0.03
Liver	5.51a	3.73b	3.94b	4.07b	0.09
Kidney	0.90	0.99	0.96	0.91	0.04
Thymus	0.38a	0.31b	0.33ab	0.35ab	0.03
Heart	0.42	0.43	0.45	0.43	0.03

Differences marked with a, b differs significantly at P<0.05

Table 38. Morphological characteristic of different parts of small intestine walls by use of faba beans
Tablica 38. Morfološke značajke različitih različitih dijelova stijenke tankog crijeva uslijed korištenja boba

(Van Leeuwen et al.,1995)

	Diet			SEM
	Control	Low tannin diet	High tannin diet	
Villus height (um)	590-415	600-448	642-454	27
Mean	515	536	565	
Crypt depth (um)	313-251	350-243	339-222	14
Mean	291	299	293	
Villus/crypt ratio	1.9-1.7	1.8-1.9	1.9-2.1	0.1
Mean	1.8	1.8	2.0	
Index of mitosis (No/100 crypt cells)	1.8-4.3	1.9-3.1	1.6-4.1	0.2
Mean	2.9	2.7	2.9	
Goblet cells (No per crypt)	24.4-20.8	25.4-20.2	23.2-21.3	0.6
Mean	22.9	23.4	22.2	
Length of microvilli (um)	1.5-1.9	1.6-2.1	1.6-1.8	0.1
Mean	1.8	1.9	1.7	
Sucrase activity (isomaltase) u/g protein)	45-61	49-78	64-86	9
Mean	53	64	63	
Aminopeptidase activity (u/g protein)	118-109	108-140	65-145	15
Mean	113	120	92	

Table 39. The effect of variety of lupin and gramine content on some biochemical indices in birds
Tablica 39. Učinak sorta lupina i sadržaja gramina na neke biokemijske pokazatelje u peradi

(Smulikowska et al.,1999)

Variety of lupin	g L/g G per kg diet	g L/g G per kg diet	Hemato-crit (l/l)	Lympho-cytes (%)	Granu-locytes (%)	Eosino-philes (%)	Uric acid in serum (mg/dL)	Creati-nine in serum (mg/dL)
Control	0/0	0/0	0.32	58.4	39.0	2.57	5.54ab	0.38
HG	200/0.2	200/0.2	0.31	64.4	34.1	2.40	5.67ab	0.37
HG	300/0.3	300/0.3	0.33	61.7	36.3	1.83	5.21a	0.34
LG	200/0	200/0	0.30	55.2	41.4	3.37	6.13ab	0.45
LG	300/0	300/0	0.31	54.5	42.8	3.20	5.51ab	0.39
LG	200/0.2	200/0.2	0.32	55.4	41.6	3.43	6.10ab	0.43
LG	300/0.3	300/0.3	0.33	61.7	35.6	3.00	7.25ab	0.40
LG	300/0.6	300/0.6	0.33	62.0	35.1	2.87	7.26ab	0.35
LG	300/1.2	300/1.2	0.33	65.7	31.5	2.67	7.98b	0.36
SEM			0.01	3.9	3.9	0.68	0.66	0.03

HG – high gramine lupin; LG – low gramine lupin; a, b – $P<0.05$

Table 40. Free plasma amino acid concentrations (mM/l) in rats fed diets containing legume globulin (G) proteins

Tablica 40. Slobodne koncentracije aminokiselina plazme u štakora hranjenim obrocima koji su sadržavali bjelančevine mahunarki /globulin (G)

(Rubio et al.,1999)

	Lacto-albumin	Lupin seeds meal	Lupin residue	Lupin G	Faba bean G	Soya G	Pooled SD
Gly	143a	272b	169a	248b	242b	252b	36.2
Ala	383a	513ac	488ac	367a	240b	175b	69.2
Val	58a	122b	50a	44a	52a	32a	18.2
Met	38a	16b	14b	13b	23ab	38a	8.3
Ileu	32a	59c	30a	13b	26ab	15b	10.4
Leu	93a	62ab	82a	23b	39b	14b	16.8
Tyr	33	44	43	30	34	24	10.8
Phen	22ab	35b	14a	13a	25ab	12a	6.9
Orn	29a	137b	43a	64a	25a	12a	20.4
Lys	496a	241b	381c	216b	283b	197b	40.2
His	15a	62b	19a	39b	41b	44b	7.9

Differences marked with a, b differs significantly at P<0.05

The numerous investigations were focused mainly on the inhibition of antinutritive action of greater amounts of lupin seeds in the diets, but today the legume ingredients are the target of studies and experiments carried out to find the potential beneficial effects of natural biologically active substances.

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SAŽETAK

Leguminoze su važan izvor hrane za široku populaciju životinja na svijetu. Obilježava ih visoka razina vrijednih mineralnih tvari, ali prije svega one predstavljaju važan izvor bjelančevina. Neke dijelove leguminoza konzumiraju i ljudi i posebno su važne u područjima gdje je konzumacija životinjskih bjelančevina iz različitih razloga ograničena. Osim dobre vrijednosti kao hrane ili krme, leguminoze su također izvor različitih štetnih (toksičnih) tvari. Treba uzeti u obzir da su sjemenke najotrovniji dijelovi biljke.

Svrha ovog članka je pokazati neke, potencijalno štetne tvari, uključene u leguminoze, mogu ispoljiti povoljne učinke u hranidbi životinja.

Ključne riječi: mahunarke, antinutritivni sastojci, kemijski sastav, kultivari

Napomena uredništva

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