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Naturally occurring toxicants in Feedstuffs and deactivation methods

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Introduction

Naturally occurring toxins in plants are the nature's defensive agents for the plants and produce deleterious effects in livestock, and known as Antinutritional factors (ANFs) (Table 1). The monogastrics behave differently from ruminants. The vegetable feed ingredients fed to monogastrics contain phytates and nonstructural polysaccharides. Supplemental phytase and exogenous enzymes is a routine practice in broiler diets. Certain enzymes are also used to supplement the naturally occurring enzymes (e.g. lipase).

A description of ANFs and methods to eliminate/minimize these in certain feed ingredients are given. The feed ingredients considered are: soybean meal, mustardseed meal, cottonseed meal, guar meal and castorseed meal.

SOYBEAN *(Glycine max)* MEAL

Soybean meal (SBM) is the principle protein source for monogastrics. Soybeans contain a high quality protein with a good amino acid profile, but for methionine, and are highly digestible in the small intestine.

Antinutritional factors (ANFS) in SBM

Soybeans contain several ANFs. The most important ANFs are described in Table 2. Some ANFs are of nutritional significance and can be **destroyed/inactivated by proper heat**

Table 1. Natural toxicants in common feedstuffs.			
Feedstuff	**Toxicant**	**Feedstuff**	**Toxicant**
Grains		**Forages –** Legumes	
All	Phytates	Alfalfa	Saponins, phytoestrogens, bloating agents
Rye, triticale	Trypsin inhibitors		
Milo	Tannins		
Grain amaranth	Oxalates, saponins	White clover	Cyanogens, phytoestrogens, bloating agents
Buckwheat	Fagopyrin		
Protein Supplements			
Soybeans	Trypsin inhibitors Lectins Goitrogens Saponins phytates	Alsike clover	Photosensitizing agents
		Sweet clover	Coumarin
		Subterranean clover	Photoestrogens
Cottonseed	Gossypol tannins cyclopropenoid fatty acids	Crown vetch	B-Nitropropanol glycosides
		Leucaena spp.	Mimosine
		Indigofera spp.	Indospecine
Rapeseed	Glucosinolates Tannins Erucic acid Sinapine	**Grasses**	
		Forage sorghums	Cyanogens
		Tall fescue	Ergot alkaloids
Linseed meal	Linatine linamarin	Tropical grasses	Oxalates saponins
Fava beans	Trypsin inhibitors Vicine, lectins	**Others**	
		Forage brassicas	Brassica anemia factor
Field beans	Trypsin inhibitors Lectins		

treatment (e.g. trypsin inhibitors) and some by **supplemental enzymes** (NSP enzymes, phytase), while others are unaffected by the methods applied now commercially.

Heat treatment inactivates or destroys the important ANFs present in SBM. The processing variables are temperature, time, moisture and particle size of the product. The different processing conditions are cooking, wave emission treatment and treatment with heated gases, roasting and extrusion.

Table 2. Antinutritional factors (ANFs) in soybeans			
Antinutrient		**Conc in Soybean**	
		Raw	**Processed**
Protease inhibitors: Kunitz factor and Bowman-Birk factors are the important inhibitors. They bind to trypsin and chymotrypsin	mg/g CP	45-60	4-8
Phyto-hemaglutins: Lectins	mg/kg	50-200	50-200
Soy antigen: Glycinin ß-conglycin	 mg/g mg/g	 150-200 50-100	 40-70 10-40
Saponins	%	0.5	0.6
Oligosaccharides	%	14	15
Stachyose	%	4-4.5	4.5-5
Raffinose	%	0.8-1	1-1.2
Phytic acid bound P	%	0.6	0.6
See van Eys et al. (2004) and others			

Tests to estimate the destruction / inactivation of ANFs

Several tests are available to estimate the destruction or inactiveness of ANFs. These may be the ones to determine the ANFs directly or involve the estimation of other enzymes or solubility property of protein, nitrogen in water or KOH solution. The latter are commonly employed to determine the ANFs in commercial channels (Table 3).

- **Trypsin inhibitors** – The principle trypsin inhibitors are the Kunitz factor and the Bowman-Birk factor. The residual trypsin inhibitor in soy products combines with the trypsin in the small intestine and forms an inactive complex thus reducing digestibility of protein. In addition to the negative effect on protein digestibility, the trypsin inhibitor induces pancreatic hypertrophy and leads therefore to an increase in secretion of trypsin (endogenous nitrogen). The combined effect on the animal is a reduction in nitrogen retention, growth and feed conversion.

The procedure described to determine trypsin inhibitors activity is based on the ability of the inhibitors to form a complex with the enzyme and thus to reduce the enzyme activity. Uninhibited trypsin catalyzes the hydrolysis of a synthetic substrate BAPNA, forming a yellow-colored product and thus producing a change in absorbance. **One trypsin unit (TIU) is arbitrarily defined as the amount of enzyme, which will increase absorbance at 410 nm by 0.01 units after 10 minutes of reaction for each 10 ml of reaction volume.**

- **Lectins** – Lectin is a protein with a specific binding affinity for sugar residues. The lectin-sugar interaction is important at the level of the membrane receptors in the gut where it is thought to be responsible for agglutination and mitosis (Pusztai, 1991). Lectins are heat sensitive and are therefore only present at residual levels in soybean products. Heat treatment to inactivate antinutritional factors in soy products is less efficient for antigens than for trypsin inhibitors or lectins (van Eys *et al.*, 2004).

The level of soy lectins can be estimated by measuring the hemaglutination activity. ELISA (total lectins) and FLIA (functional lectins) tests are more sensitive and selective (Delort-Laval, 1991).

- **Soy antigens** – Immunoassay techniques are used to determine concentrations of soy antigens (glycinin and ß-conglycinin) in soy products. The level of glycinin and ß-conglycinin can be measured by a specific competitive inhibition ELISA using anti-soy globulin Pabs (Heppell *et al.*, 1987).

The indirect methods employed are the urease index, protein solubility in KOH and protein and nitrogen dispersability.

- **Urease Index** – Both the enzymes, urease and trypsin inhibitor, are deactivated during heating. Instead of estimating the trypsin inhibitor directly, urease activity is measured as an indicator to assess whether the anti-nutritional factors, such as trypsin inhibitors, present in soybeans have been destroyed by heat processing.

Indirect methods to test the adequacy of heat treatment

The urease index is the most common test used to evaluate the quality of the soybean processing treatment. The optimum pH increase is considered to be between 0.05 (McNaughton *et al.*, 1980) and 0.20 (Waldroup *et al.*, 1985). Usually, all overheated samples yield urease indexes below 0.05, but that does not imply that all samples with urease tests below 0.05 have been overheated. It is recommended that, when using soybean products for swine or poultry the increase in pH is not greater than 0.35 (Waldroup *et al.*, 1985). Animal performance is severely impaired with urease indexes above 1.75 pH units.

Table 3. Indirect methods to estimate the destruction of antinutritional factors (ANFs) in soybean meals

Method to estimate the destruction of antinutrients		Conc in Soybean	
		Raw	Processed
Urease index Δ	pH	2.5	< 0.30
Protein solubility in KOH	%	97	70-85
Protein dispersability index (PDI)	%	92	15-30
Nitrogen solubility index (NSI)	%		10-11
Saponins	%	0.5	0.6

- **KOH Protein solubility** – This method consists of determining the percentage of protein that is solubilized in a potassium hydroxide (KOH) solution (Araba and Dale, 1990). The KOH protein solubility is not sensitive enough to gauge the level of heat processing that a soybean product has undergone, but it is effective in differentiating overheated products from correctly processed ones.
- **Protein Dispersability Index (PDI) and Nitrogen solubility Index (NSI)** – The PDI is the simplest, most consistent, and most sensitive method. This test measures the solubility of soybean proteins in water and is probably the best adapted to all soy products. The PDI method measures the amount of soy protein dispersed in water after blending a sample with water in a high-speed blender. The water solubility of soybean protein can also be measured with a technique called Nitrogen Solubility Index (NSI).The two methods differ in the speed and vigor at which the water containing the soybean product is stirred. In animal nutrition the PDI method is used.

ANFs not destroyed by heat – The important ANFs that are not destroyed by heat are the phytates and oligosaccharides.

COTTONSEED *(Gossypium Spp)* MEAL

The cottonseed contains: hull 23%, oil 17%, linters 8% and meal 52%. Cotton seed meal un-decorticated and decorticated (expeller and solvent extracted) and cottonseed hull are used in the concentrate mix for ruminants and Cotton seed meal decorticated and deoiled in the diets for monogastrics. Cottonseed meal un-decorticated contains about 25% protein and the decorticated one about 42% protein.

Cotton seed meal protein is low in three important essential amino acids, lysine, methionine and threonine.

Antinutritional factors (ANFs) in cotton seed meal

The Cotton seed meal contains ANFs: High fibre, low protein quality, toxic components: gossypol and cyclopropenoid fatty acids.

Gossypol is a polyphenolic yellow compound present exclusively within discrete bodies called 'pigment glands' in leaves, stems, roots and endosperm of seeds). In unprocessed state, entire gossypol is in free state (FG). The gossypol may be levorotatory and dextrorotatory. Due to processing, some gossypol is bound to protein or free amino-groups of amino acids (BG). Information is not available whether bound gossypol is converted to free gossypol within the intestinal tract of monogastrics.

The FG and BG content of cottonseed cake vary with the type of processing (Table 4).

a. **Dehulling increases gossypol concentration in the cake.**

b. **Solvent extraction increases** FG content (than expeller processing). The two

reactive carbonyl groups of gossypol react with free amino groups of basic amino acids, particularly of ε-amino group of lysine.

2. Cyclopropenoid fatty acids (CPFA)

The cyclopropenoid fatty acids (CPFA: malvalic and sterculic acids) are the components of residual oil (0.01% in meal obtained by commercial practices of extraction), increase the permeability of the vitelline membrane for iron of yolk to diffuse into the albumen, resulting in the formation of red iron-albumen complex accounting for pink white in stored eggs.

Toxic effects of feeding cottonseed meal – The signs of gossypol toxicity in poultry are decreased appetite, weight loss, leg weakness, decreased egg size, egg yolk discoloration, reduced hatchability, lowering of hemoglobin, total erythrocytic count. FG acts by reducing oxygen carrying capacity of the blood (Alford *et al.*, 1996), may adversely affect male reproductive function (testicular damage) (Randel *et al.*, 1996).

The safe level of FG is 50 ppm for layers and 100-150 ppm for broilers (Waldroup. 1981: Martin (www.cottonseed.com). 1970). The NOAEL and LOAEL dose for different animals is given in Table 5 (See Scientific Opinion, 2008).

Cotton seed meal in Poultry diets – Cotton seed meal solvent extracted was tolerated up to 8 to 30% in diets for chicks. Supplemental iron (600 mg/kg; El Boushy *et al.*, 1989) and lysine proved beneficial in supporting the growth of chicks (Campbell, 1988). CPFA in residual cottonseed oil have been shown to cause deposition of increased amounts of saturated fatty acids in egg yolks due to inhibition of desaturase enzymes (Reid, 1972).

EU legislation is given in Table 6 on FG of feed materials used for livestock, poultry and aqua.

Detoxification of Cotton seed meal – Chemical treatment with iron salts, solvent extraction of Cotton seed meal and development of glandless Cotton seed meal, fermentation with fungi are some possibilities to reduce the toxicity of gossypol of Cotton seed meal.

Vitamin E was beneficial in alleviating the effects of FG in dairy bulls.

Mustardseed *(Brassica Sps)* Meal

Both the mustard *(Brassica juncea)* and rapeseed *(Brassica napus)* belong to the Brassica family. Canola, developed by plant breeders recently, is a

Table 4. Gossypol (Free and total) in various types of Cotton seed meal (mg/kg)

Type of CSM	FG	TG	Reference
Decorticated Exp CSM	360	6920	Sharma, 1978
Undecorticated CSM	2700	5183	Nagalakshmi et al., 2002
Glandless CSM	106	362	Ryan et al., 1986
CSM=Cotton seed meal			

Table 5. Free gossypol – No observable adverse effect level (NOAEL) and lowest observable adverse effect level (LOAEL)

	NOAEL		LOAEL	
	mg/kg diet	mg/kg BW/day	mg/kg diet	mg/kg BW/day
Calves	200	4-5		
Lamb				2-3
Pig		3		
Broiler	200	20-30		
Fish			140	
Gossypol is transferred to edible tissues including muscle, offal of ruminants, poultry, fish, and into eggs and probably cow's milk (Scientific opinion 2008).				

Table 6. EU legislation on free gossypol containing plant materials used as feed	
Product intended for animal feed	Maximum content relative to a feeding stuff with a moisture content of 12 %, mg/kg
Feed materials with the exception of:	20
- cottonseed	5000
- cottonseed cakes and cottonseed meal	1200
Complete feedingstuffs with the exception of:	20
- complete feedingstuffs for cattle, sheep, goats	500
- complete feedingstuffs for poultry (except laying hens) and calves	100
- complete feedingstuffs for rabbits and pigs (except piglets)	60
Scientific opinion, 2008.	

double zero variety for glucosinolates and erucic acid.

Mustard meal is a fairly good source of crude protein (36%), lysine, methionine and energy. The ANFs in mustard meal are: (i) glucosinolates, (ii) erucic acid, (iii) high fibre, (iv) NSPs. Besides these toxic principles, orgemone seed is a frequent contaminant in mustard cake.

About 120 different glucosinolates (Gls) are known to occur naturally in plants. Brassica sps contain the glucosinolates. The Gls share a common structure comprising a β-d-thioglucose group, a sulphonated oxime moiety and a variable side-chain derived from methionine, tryptophan or phenylalanine (See Tripathi and Mishra, 2007).

The major deleterious effects of glucosinolates ingestion in animals are reduced palatability, growth, production and egg weight, deranged liver and kidney functions, interference with iodine availability and morphological and physiological changes of thyroid. Deleterious effects of Gls are greater in non-ruminant animals compared to ruminants. In general, young animals are more sensitive to Gls than adult and older animals (See Trpathi and Mishra, 2007).

Broilers – Many workers (Vaidya *et al.*, 1979, Mandal *et al.*, 1981; Prasad and Rao, 1982a) noticed an increase in weight of thyroid gland, liver and fat content of liver fat due to feeding 20 - 30% MC, attributed to the erucic acid content of MC (Prasad and Rao, 1982a). The toxic effects of intrinsic principles present in mustard cake decreased with age of the bird. Expeller processed MC depressed the body weight gain when incorporated beyond 14% in WL chick diet (21 and 28%) at 8 weeks; However, the body weight gain of chicks fed 28% MC at the latter age was similar to those fed ground nut meal control diet (Jagdish Prasad *et al.*, 1973). Low glucosinolate CMC (Carboxy Methyl Cellulase) can be used as the sole protein source up to 53.5% and the conventional CMC at 21.5% in broiler diets (Rama Rao *et al.*, 2005).

Prasad and Rao, (1982a) concluded that erucic acid is the main performance inhibitor than tannins in MC for WL starter chicks. Erucic acid at levels higher than 1.2% in chick diet significantly depressed weight gains and feed efficiency. Similarly, in WL cockerel diet (10-20 weeks) expeller pressed MC can be incorporated up to 20% replacing ground nut meal (w/w) without affecting weight gain and feed efficiency (Jagadish Prasad *et al.*, 1974). However, enlargement of thyroid gland and increased weights of testis and kidney were the detrimental affects noticed in MC fed birds. Solvent extracted MC can be used as a total replacer for ground nut meal (25%) on protein basis in WL chick (1d to 15 weeks) diet without affecting body weight gain, feed efficiency and hematological parameters (Mandal *et al.*, 1981).

Layers – In layers, inclusion of MC in place of groundnut meal and fishmeal at 10% level in iso-caloric and iso-nitrogenous diets significantly reduced the egg production and feed efficiency (Shrivastava *et al.*, 1976; Mandal and Saxena, 1985). Iodine supplementation failed to improve

egg production in birds fed MC based diet (Lodhi *et al.*, 1978). Contrary to these findings several workers (Sadagopan *et al.*, 1983) found no adverse effect on egg production, egg weight, feed efficiency, and body weight in layers fed with diets containing 10-16% expeller or solvent extracted MC.

Shell thickness, shell weight and yolk index were unaffected with MC inclusion (Shrivastava *et al.*, 1976, Sadagopan *et al.*, 1983), while, HU score and albumen index were significantly reduced in diets containing high levels of MC. Eggs from hens consumed 15 - 20% MC in their diet scored lower acceptance when they were stored under refrigeration for 14 days. Utilization of MC in layer diet (brown shelled layers) may result in production of fishy or off-taint eggs. The fishy taint was due to the presence of excessive amount of trimethyl amine (TMA) in the yolk. The excessive accumulation of TMA is resultant of destruction of TMA-oxidase by glucosinaltes and tannins present in MC.

Castor Seed *(Ricinus communis)* Meal

Castor seed meal contains 30-40% protein. Castor seed meal contains the ANF "Ricin". Ricin was found to have high haemoagglutinating and proteolytic activity (Liener, 1980). The Castor seed meal contains ricin to an extent of 0.22-1.0%. The Castor seed meal also has allergen, ricinine and chlorogenic acid but toxicity is only due to the presence of ricin. The type of solvent used for oil extraction has influence on level of toxins and toxicity (Ambedkar and Dole, 1987)

Different physical (dry or moist heat) and chemical methods have been tried to detoxify the Castor seed meal. Dry heating (140°C) for 20-90 minutes was found to be effective in removal of toxic principles in Castor seed meal. Lime treatment (0.25% w/w) followed by extrusion cooking has been proved to be the most effective detoxification method developed through NATP (Anonymous, 2005). Treatment of cake with solvents like ethyl alcohol and chloroform detoxify the cake to a great extent, but the processes are not feasible and economical on large scale. It can be rendered suitable for animal consumption by treatment with proteolytic enzymes, autolyzed yeast and Azetobacter.

Mahua Seed Meal

Mahua *(Madhuca indica, M. longifolia)* seed meal is a moderate source of protein (15-24%). Mahua seed meal contains saponins: Madlongiside A, B, C, D; Mi-saponin A (Yoshikawa *et al.*, 2000). The saponins are having hemolytic effect. Mahua cake in chick mash at about 12% in feed caused mortality of all chicks within five hours (AICPR, 1979, cited in Jakhmola *et al.*, 1987). Even at 5%, mahua seed meal depressed the performance of chicks (Ramesh Kumar *et al.*, 2000). Ethanol treatment of mahua seed meal reduced 86.46% saponins and 31.77% tannins (Singh and Agarwala, 1989). An excellent account is available of saponins in Madhuca longifolia L. as undesirable substances in animal feed is available in scientific opinion, 2009.

Guar *(Cyamopsis tetragonaloba L.)* Meal

Guar meal, a protein source, causes deleterious effects (Nagpal *et al.*, 1971, Shingari and Ichhponani, 1976, Pradhan, 1978) in poultry, because of residual gum. Guar meal can be incorporated up to 8% in commercial broiler (1 to 6 weeks) diet. The higher levels of inclusion (16 and 32%) of guar meal caused depression in growth and feed efficiency, and sticky diarrhea and high mortality (25 to 70%) (Nagra *et al.*, 1985). Residual gum (18 to 21%) present in guar meal (Nagpal *et al.*, 1971, Prasad *et al.*, 1981, Nagra, 1982) may be responsible for deleterious effects in broilers. Trypsin inhibitors, (Nagra *et al.*, 1985), tannins (Nagra, 1982) and saponins (Kaur,

1981) may be the contributing factors for guar meal toxicity. Raw guar meal in chick diet (6 weeks of age) at 15% resulted in degenerative and necrotic changes in the kidney and intestine (Singh *et al.*, 1992). Toasted guar meal in diets (5 to 20%) depressed the energy utilization in broilers (1 to 10 weeks). However, the deleterious effects of toasted guar meal on energy utilization was completely overcome by supplementation of ground nut oil (8.2 to 9.9%) (Prasad *et al.*, 1981). Guar meal when fed as a sole source of protein (40% of diet) reduced the digestibility of crude protein, fat and carbohydrates and caused severe mortality, enlargement of pancreas, liver and gall bladder, and atrophy of spleen (Nagpal *et al.*, 1971). The ill effects of guar meal on broiler (1 to 70 d) performance partly can be alleviated with higher dietary protein (20 vs 30%) content (Thakur and Pradhan, 1975).

Guar meal can be incorporated in WL grower diet and layer diets up to 10 and 11%, respectively (Nagra and Virk, 1986). The depression in egg production may be corrected by increasing the dietary protein (Saxena and Pradhan, 1974).

ABSTRACT

Antinutritional factors (ANFs) present in many of the vegetable feed ingredients limit the inclusion of these ingredients for monogastrics, aqua and livestock. The important vegetable feed ingredients for animal nutrition are soybean meal, mustard seed meal, cotton seed meal, castor seed meal and guar meal. All these ingredients contain ANFs and cannot be used at fairly high levels in the diets.

Soybean meal is the principle protein ingredient for poultry and aqua. Soya seeds contain several ANFs, the most important being the trypsin inhibitors. Soya seeds are subjected for heat treatment as a routine method to limit the trypsin inhibitors and other heat susceptible ANFs. The ANFs present in soybeans not susceptible for heat treatment do not generally create problems in monogastric production practices, when soybean meal is used as the sole source of protein. Cotton seed meal contains gossypol (gossypol binds with free amino groups of amino acids/protein) and cyclopropenoid fatty acids. The inclusion in poultry diets is very much limited because of high variability in free gossypol content, depression in productivity and egg discoloration. Inclusion of mustard meal is limited in poultry diets due to the presence of glucosinolates. Research in the inclusion of mustard meal in poultry suggested higher levels of inclusion; however in practice the inclusion is restricted up to 4-5% for growth and egg production. Guar meal is being included in poultry diets but at a very low inclusion level (2-4%), due to the variability in the guar gum content and the processing of toasting employed.

ANFs are the natural protective substances for the plant. It is possible that at a very low substitution, the ANFs of the vegetable protein sources such as guar meal, castor seed meal and mahua meal, etc might have a nutraceutical function or immunological function. Future research may be directed on this also while evaluating any vegetable protein source containing ANFs.

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