Advances in Dairy Research
ISSN: 2329-888X

Tripathi et al., J Adv Dairy Res 2017, 5:3
DOI: 10.4172/2329-888X.1000180

Research Article Open Access

Prospects and Problems of Dietary Glucosinolates in Animal Feeding

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Received date: 10 November, 2016; **Accepted date:** 08 July, 2017; **Published date:** 17 July, 2017

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Abstract

Brassica originated feed and fodders are the chief source of glucosinolates in animal diets. High intake of glucosinolates induce several health and production problem in animals, however low glucosinolates containing meals may be good source of protein especially of sulfur containing amino acids. Recent research on glucosinolates shown that feeding of low glucosinolates containing meals reduce fungus infestation of ration, increase isothiocynate content of animal produce and increased peripheral fat with higher contents of unsaturated fatty acids ($C_{18:00, 1\ trans}$, $C_{22:2}$) in carcass and milk fat, are advantages of *Brassica* meal feeding because isothiocyantes and unsaturated fatty acids exerts anti-carcinogenic properties. The deleterious effects of high glucosinolate containing meals could be eliminated using a suitable detoxification technology and keeping the level of glucosinolate within tolerable limits.

Keywords: *Brassica* meals; Glucosinolates; Product quality

Introduction

The glucosinolates are a large group of sulphur-containing secondary plant metabolites, which occur in all the economically important varieties of *Brassica*. A wide variety of glucosinolates exists owing to modification of the side-chain structure. To date more than 120 different glucosinolates have been identified, all shares a common structure comprises a β-D-thioglucose group, a sulphonated oxime moiety and a variable side-chain derived from methionine, tryptophan or phenylalanine [1]. Dietary glucosinolate upon ingestion undergo microbial bio-conversion into different bioactive metabolites [1,2]. These bio-active metabolites produce health and productive problems on different animals. The negative effect of glucosinolates varies depending on the type and level of glucosinolates present in meal. Non-ruminants are more sensitive to dietary glucosinolates than occur in ruminants, and young animals are severely affected than adult animals [1,3,4]. Health and production effects induced by dietary glucosinolates include depressed growth and production, reduced palatability and intake, hypothyroidism/metabolic and other disturbances. Through, genetic manipulation new double low *Brassica* cultivars are evolved [5,6], these cultivars contain very low concentrations of antinutritional substances glucosinolates and erucic acid, are referred as canola. Standards of double low cultivars differ between countries to countries. This complicates the levels of rapeseed meals inclusion in feeding. According to Swedish specification for 00 RSM, erucic acid should not account for more than 2% of the total fatty acid and each gram fat free dry matter should not contain more than 40 µmoles total glucosinolates. The Canadian standards are similar for erucic acid but the glucosinolate limit is less than 30 µmoles/ g fat free DM, which does not include indolyl-glucosinolates [1,5]. The European Community countries allow a limit for glucosinolates per gram air dried rapeseed is 20 µmoles, including indolyl-glucosinolates.

Glucosinolate Sources and Metabolism

Rapeseed meal is the chief source of glucosinolates (Gls) and it is a good source of protein and sulfur containing amino acids in animal nutrition. Content and composition of Gls varies due to plant species, agronomic practices and climatic conditions. The Gls content is generally higher among rapeseed meal (RSM) produced under tropical environment than occur in temperate. The glucosinolate composition is also influenced by growing conditions, the RSM originated from Indian sub-continent contain primarily 3-butenyl, 2-propenyl and 4-pentenyl glucosinolates in proportion of 73, 24 and 1% respectively, other indoyl and aromatic glucosinolates are in the range of 1.6 to 3.7%, whereas glucosinolates from European and other temperate countries contain basically 2-propenyl glucosinolates, accounting to more than 95% of their total glucosinolates and did not contain 4-pentenyl glucosinolates. Fermentation conditions (pH and cofactors), Gls content and composition of RSM effects the level and type of metabolites, major metabolites are thiocyanates (SCN), isothiocyanates (ITC), nitriles, 5-vinyl-2-oxazolidinethione (VOT) and 5-vinyl-1,3-oxyzolodine-2-thione (5-VOT). Reduced palatability, decreased growth and production are the major deleterious effects of glucosinolate ingestion in animal diets. Apart from total glucosinolate content SCN, nitriles and VOT estimates are the chief attribute of RSM quality as these are produced upon hydrolysis of Gls following the processing of RSM. Progoitrin and epi-progoitrin content is the palatability attributes, palatability impairs at level between 2.3 to 4.65 µmole g^{-1} diets, while at higher a significant feed intake depression occurs. Nitriles are known to affect liver and kidney functions. The SCN interferes with iodine availability, whereas 5-VOT is responsible for the morphological and physiological changes of thyroid. Difference in Gls profile among the RSM induces varying levels of glucosinolates metabolites in animal tissues. The indoyl glucosinolates, precursors of thiocyanates will increase the SCN concentration whereas, corresponding intake of progoitrin (2-hydroxy-3-butenyl glucosinolate), will increase the concentration of 5-VOT. High intake of glucosinolate progoitrin induces an accumulation of 5-VOT mainly in thyroid and lungs. The 5-VOT has a high affinity for thyroid. The SCN are secreted in milk of RSM fed animals. RSM feeding did not

impairs quality traits of carcass, whereas RSM feeding results a higher contents of unsaturated fatty acids ($C_{18:00, 1\ trans}$, $C_{22:2}$) in carcass and milk fat [1].

Glucosinolate Tolerance

Ruminants are less sensitive to dietary glucosinolates than non-ruminants (Table 1). *Brassica juncea* meal with 72.58 mol Gls/g DM could be incorporated in the concentrate mixtures up to 225 g/kg and lactating goats can tolerate a daily intake of Gls 11.76 mmole, which amounts 9.45 µmol Gls/g diet DM [7]. Inclusion of *Brassica* derived products in diets of dairy animals are known to increase the nutraceutical properties of milk [8] and milk products [9]. Among, non-ruminants, pigs are more severely affected by dietary glucosinolate compared to rabbit, poultry and fish. Young ruminants can tolerate total Gls up to 4.22 µmole g^{-1} diet, whereas pig 0.78, rabbits 7.0, poultry 5.4 and fish 3.6 µmole g^{-1} diet. Among various treatments to detoxify RSM; water extraction, heat and $CuSO_4$ treatments were suitable for RSM quality improvement. Added iodine supplementation 1.0 mg kg^{-1} diet in pigs and 500 mg I each kg RSM included in ruminants diets seems promising because of economic and easiness compared to other treatments. Therefore, a desired amount of RSM can be used for animal feed formulation by adopting a suitable technology to minimize or remove Gls related deleterious effects on animals. Additionally, increased contents of unsaturated fatty acids ($C_{18:00, 1\ trans}$, $C_{22:2}$) in carcass and milk fat and deposition of ally-isothiocynate (AITC) are advantages of RSM feeding because of their human health promoting effects.

Technologies to Reduces Glucosinolate Level

Among techniques worked out to remove glucosinolate and minimise the deleterious effect on animals heating, pulping and solid-state fermentation were found effective (Table 2). Heating reduces glucosinolates depending on the type of compound, degree and time of heating; the degradation products are hardly detectable, except of thiocyanate ions and ascorbigen. Pulping resulted in complete breakdown of glucosinolates by autolysis, glucosinolates may partly survive depending on the degree of crushing [10]. The degradation products isothiocyanates and nitriles are persisting during pulping. The fermentation gradually decreases the glucosinolates level to zero, where the degradation products 1-cyno-3-methylsulphinylpropane may remain in the final product. Processing of glucosinolates containing food/feed give rise to a certain degree of glucosinolates breakdown products by enzymatic hydrolysis or other chemical reactions. The glucosinolates degradation takes place at each level of feed processing starting from oil extraction to diet preparations. Isothiocyanates produced during processing or fermentation may either absorb by the intestinal epithelium or under certain circumstances isothiocyanates undergo rearrangement reactions to form thiourea or corresponding amines. Allyl-isothiocyanates formes allyl-amine whereas benzyl-isothiocyanates formes benzyl-amine. Free amines formed by rearrangement [11] reactions of isothiocyanates may undergo further chemical changes to yield thiourea by reaction with n-butyl-amine. Thiourea also known to induce hypothyroidism in small ruminants.

Biological Effects

Glucosinolate themselves are biologically inactive molecules; however glucosinolates degradation products are biologically active and known to their diversified biological effects. Negative effects of glucosinolates on animals are relative to its concentration in diet. Isothiocyanates, although responsible for bitterness but considered to exert health promoting effects, whereas nitriles exert health degrading influence. Thiocyanates, thiourea and oxazolidithione may disrupt iodine availability to thyroid thus affecting thyroid function [12]. Glucosinlate metabolites are also responsible for the morphological and physiological changes of thyroid, goitrogenecity, mutagenecity, hepatotoxicity and nephrotoxicity [13,14]. The negative influence of dietary glucosinolate on animal growth and production are due to drastic endocrine disturbance-induced [15] by different glucosinolate metabolites.

Bio-conversion of Glucosinolates

A wide variety of glucosinolates exists owing to modification of the side-chain structure and to date more than 120 different glucosinolates have been identified. Glucosinolate content and composition varies by the plant species, agronomic and climatic conditions. Glucosinolate content of temperate mustard (*Brassica juncea*) is quite high but mainly of sinigrin (90%) and glucotropeolin (10%). The total glucosinolate content may be up to 169 µmol/g seed dry matter. Glucosinolates upon ingestion undergo microbial bio-conversion into bio-active health promoting isothiocynates depending on the predominate bacterial strains in gastrointestinal tract. Accordingly, bioconversion rate varies from 20 to 95% due to large variation among individual intestinal flora. Therefore, different bacterial strains have varying capability to produce myrosinase activity for glucosinolate bio-conversion. Integration of glucosinolate bioconversion data with predominate intestinal bacterial groups with individualflora revealed that *Bacteroids* and *Bifidobacterium* groups possess higher ability to produce microbial myrosinase, thereby higher glucosinolate bioconversion efficiency. Increased population of *Clostridiun leptum* group suppresses the glucosinolates bioconversion efficiency of *Bacteroids* and *Bifidobacterium* groups. Whereas, *Clostridium coccoides*, *Atopobium*, *Lactobacillus* and *Enterobacteria* groups influence myrosinase activity of intestinal flora [16]. The glucosinolate bioconversion completes within 36 hr of incubation by microbial myrosinase. When plant myrosinase is active the pH of the culture medium influences and get decline toward acidic which favor nitrile production. Higher gas production under plant myrosinase inactivated samples indicated that plant myrosinase not only affects glucosinolates bioconversion but it alters entire microbial fermentation.

Animal species	Glucosinolates level (maximum upper limit)	Reference
Rat	0.5 µmol per gram diet	[16]
Pig	0.78 µmol per gram diet	[17]
	2 µmol per gram diet with added supplementation of at least 1000 µg Iodine/kg diet	[18]
Poultry	2-4 µmol per gram diet	[19]
Rabbit meat	7 µmole per gram diet	[20]
Growing steers	10 to 15 µmol per gram diet	[21]
Growing calves	7.7 µmol per gram diet	[19]
Dairy cow	11 µmol per gram diet	[22]

Goat	16 µmol per gram diet	[7]
Young lamb	2 µmol per gram dry diet	[23]
	0.88 µmol per gram liquid diet	[24]
Fish	1.4 µmol per gram diet	[25,26]

Table 1: Glucosinolate tolerance level in different animals.

Treatment	Conditions of treatment	Animal Response	Reference
Cu treatment	1 kg RSM soaked with 6.25 g $CuSO_4.5H_2O$ in 0.5 lit hot water	Pigs performance improved terms of intake growth and thyroid hormones status.	[27]
Water soaking	Seed to water ratio; 1:10 for 6 hr and dried at 60ºC	Improved intake and nutrient utilisation in sheep	[28]
	Soaking of meal for 24 h, with 2 parts of water and drying at 60ºC	Did not improve any attribute significantly in growing pig.	[29]
Water extraction	Seed to water ratio; 1:8	Detoxified meal improved animal performance	[11]
	Meal was soaked in water, 1:10 ratio, for 10 min filtered with gooch funnel at 25 psi	Not determined	[30]
Heat treatment	Toasting at 100°C, 2 h	120 min although reduced most of glucosinolates but lowered rat performance. For non-ruminant feeding 30 min toasting is optimum for 00 RSM to remove half glucosinolates and maintain protein quality	[31]
	110°C, 2 h	No significant effect of treatments on milk yield and goitrogenic substance secretion in milk	[10]
	The acid treatment for 72 hr (16 ml HCl kg^{-1} meal) and heating at 180ºC for 2 h	Acid treatment followed by heating improved nutrient utilisation and growth in calves	[32]
Heat (toasting)	103-107°C, 30-40 min.	Poultry : Reduced amino acid digestibility	[33]
Formaldehyde treatment	0.8 g/100 g CP	No significant effect of treatments on milk yield and goitrogenic substance secretion in milk	[10]
Micronization (Heat)	195°C, 90 seconds	Growing Pig : Improved nutrient digestibility	[34]

Table 2: Effect of processing methods for glucosinolate decomposition on animal's responses.

Conclusion and Recommendation

The total glucosinolate content should be taken in to consideration for RSM use in feed formulations. Estimation of Gls derived metabolites in RSM may provide added information with regard its quality and possible influence on animals. A very low glucosinolate (8-10 µmole g^{-1}) rapeseed meal can be included in pig diets up to 12%, the inclusion of meal should maintain total glucosinolate less than 0.78 µmole g^{-1} diet. Pig diets may contain glucosinolates up to 2 µmole g^{-1} but it requires 1 mg added Iodine per kg diet. A 10% RSM can be included in broiler rabbits feeding and the total glucosinolate should not exceed to 7.0 µmole g^{-1} diet. Broiler chicken diets may contain glucosinolate 6 to 10 µmol g^{-1} diet. The rapeseed meal containing glucosinolates 3.6 µmole/g can effectively replace 30% dietary protein in fish diets. An inclusion of 25% low glucosinolate rapeseed meal (15 to 33 µmole g^{-1}) contributing a total glucosinolate content 1.5 to 4.22 µmole g^{-1} diet can be possible in growing and fattening lamb diets. Diets with Gls 1.2 to 1.6 µmoles g^{-1} DM shall induce reproductive disturbance in ewes. Among various technologies developed to improve glucosinolate related deleterious effects of RSM, water extraction, the heat and $CuSO_4$ treatments seem effective. Added iodine supplementation 1.0 mg kg^{-1} in pig diets and 500 mg I kg RSM inclusion in ruminant is another effective strategy for effective utilization of RSM.

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