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GLUCOSINOLATES IN RAPE AND CAMELINA: COMPOSITION, CONCENTRATIONS, TOXICITY AND ANTI-NUTRITIVE EFFECTS IN POULTRY, METHODS OF NEUTRALIZATION — A MINI-REVIEW

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Abstract

During the last decades animal nutrition and feed production in the World encounter the increasingly important problem of the deficit of feed-grade protein especially urgent for the regions (including Russian Federation) where the soybeans (considered an "ideal" protein source in feeds for all animal and poultry species) cannot be effectively cultivated. To solve this problem, decrease feeding costs and dependence on the imported soybeans the local vegetable protein sources are increasingly used including *Brassicaceae* oil crops rape (*Brassica napus* L.) and camelina (*Camelina sativa* L.) which are highly tolerant to the conditions of cultivation. Though cakes and meals of these crops are rich in protein they contain certain anti-nutritive factors including glycosides called glucosinolates (GLs), a vast group of protective secondary plant metabolites, alkyl-aldoxime O-sulphates containing the residue of β-D-thioglucopyranoside bonded to the hydroximine carbon in *cis*-position to the sulphate group. At present over 120 natural GLs are identified (B.A. Halkier and J. Gershenzon, 2006). The toxic and anti-nutritive effects of the GLs in rapeseed and methods for their neutralization are relatively well studied; however, the effects of GLs in camelina are still understudied (due to its relatively short history of large-scale cultivation) and hence the data obtained on rape should be used for the assessment of possible effects of camelina. The detrimental biological effects of the GLs on poultry, their mechanisms and methods of neutralization are reviewed herein. The GLs per se are non-toxic and their protective role in the plants is related to the endogenous plant enzymes β-thioglucosidases (myrosinases): GLs and myrosinases normally (in an intact plant) localized in different tissues contact after the damage of the plant (e.g. by insects or other herbivores) resulting in the enzymatic hydrolysis of the GLs and transformation of their aglycone residues into the potentially toxic products: isothiocyanates, thiocyanates, oxazolydine-thiones, nitriles, epithionitriles (D.J. Kliebenstein et al., 2005). Similar processes could be also induced by the enzymes of intestinal microbiota in poultry. Main toxic effect of almost all these products is goitrogenicity involving disturbance of the synthesis and secretion of thyroid hormones into the bloodstream and (in cases of heavier exposure) hypertrophy of the thyroid gland and formation of the goiter. In sub-toxic doses these GL metabolites can hamper the growth in young poultry, decrease egg production and quality in adult hens, induce "fishy taint" of the eggs. Since 1960s the rape has been intensively selected for decreased GL content and a wide range of low-GL cultivars are now present in the market; similar work with the camelina is still at its start. Concentrations of the GLs in cakes and meals of these crops can be decreased by thermal treatment (at ca. 100 °C), soaking in water, treatments with solutions of alkali or copper sulphate, solid-phase microbial fermentation, micronization, extrusion, etc. (M.K. Tripathi and A.S. Mishra, 2007). Maximal permissible level of the GLs in diets for poultry is apparently 5-6 mM/kg, corresponding to dietary levels of the products of the native rape ca. 10 %, low-GL rape varieties 15-20 %, camelina products 5-10 %. The studies on the toxic and anti-nutritive effects of rape and especially camelina are necessary for the practice of poultry nutrition and important for further genetic improvement of these crops.

Keywords: poultry, nutrition, rape, camelina, cakes, meals, glucosinolates, goitrogenicity, selection

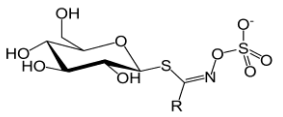
In the last two decades, due to growth of population, consumption and livestock production, feed protein deficiency has become an increasingly pressing global problem, especially in regions where soybeans, an ideal source of feed pro-

tein for animals and poultry, are not grown. In Russia, domestic soybean production is low, therefore, feed protein deficit is also relevant. To reduce the cost of feed and import dependence, domestic poultry farming uses local feed resources, in particular rapeseed (*Brassica napus* L.) and camelina (*Camelina sativa* L.) of the *Brassicaceae* family. They are unpretentious and highly productive even in the most unfavorable conditions, and are resistant to most pathogens [1, 2].

Cake and meal, the by-products after extracting oil from camelina and rapeseed seeds, are rich in protein, but contain anti-nutritional factors, primarily glycosides glucosinolates (GL) [1, 2]. This fact has been known for quite some time, but is often underestimated in diet formulation. In addition, anti-nutritional properties have been studied mainly in rapeseed, while camelina has been cultivated relatively recently compared to rapeseed and has been less studied as a fodder crop.

Antinutrients of *B. napus* have become less acute issue due to created low-glycoside canola varieties. Nevertheless, study of rapeseed remains relevant in terms of its improving as a forage crop and clarifying protocols for its use. In addition, these data partially compensate for the lack of information on camelina.

This mini-review consideres toxic and antinutritive property of glucosinolates on the example of poultry, the mechanisms of toxic action and methods for its neutralization.



Geeneral formula of glucosinolates. R is a side chain.

Biodiversity of glucosinolates and their content in rapeseed and camelina. In chemical structure, GLs are a broad class of O-sulfates of alkylaldoximes, in which a β-D-thioglucopyranoside residue is attached to the hydroxymine carbon atom in the cis position to the sulfate group (Fig.). The diversity of GLs derived from differences in the side chain (R) structure. Currently, more than 120 natural compounds of this class have been identified [3, 4].

GLs are not toxic, and their protective action is due to the endogenous enzymes β-thioglucosidases, also known as myrosinases, of the same plants. In an intact plant, they can be expressed in various tissues. When damaged, for example, by insects or animals, the enzyme and its substrate (GL) interact, the GL hydrolysis occurs with the release of glucose. Next, the aglycone residue of GL is transformed into various toxic products, e.g., isothiocyanates, thiocyanates, oxazolidinethiones, nitriles and epithionitriles. The type of substrate (GL), pH, the presence of iron ions and/or epithiospecific protein determine the ratio of these hydrolysis products [4-7]. The same process of enzymatic hydrolysis of GL by myrosinases can occur, depending on the technology conditions, during oil extraction. The resulting toxic compounds, primarily isothiocyanates, as the most thermostable products of GL hydrolysis, accumulate in cakes and meals [8]. To a certain extent, the same GL hydrolysis can occur in the digestive system of animals and poultry due to microbial transformations [9].

In the organs of the same plant, GLs with different side chains may predominate. Thus, in rapeseeds the main GLs are progoitrin (2-hydroxy-3-butenyl-glucosinolate) and gluconapine (3-butenyl-glucosinolate), and in the vegetative parts and especially in the roots glucobrassicanapine (4-pentenyl-glucosinolate), glucobrassicin (3-indolylmethyl glucosinolate) and 4-hydroxyglucobrassicin, gluconasturcine (2-phenylethyl glucosinolate) [10, 11]. Moreover, these GLs not only differ structurally, but, apparently, perform different functions. GLs of seeds are responsible for protecting the offspring, while GLs of vegetative parts are responsible for protecting the territory. Remaining in the soil, they are hydrolyzed, mainly to isothiocyanates, and have allelopathic phytotoxic effects on other plants [12]. It is precisely this effect that is associated with yield decrease in the crops followed rapeseed in crop rotation. It has been reported, for example, that soybeans

and sunflowers in fields after rapeseed were severely retarded in early growth, which did not occur in barley or when soybeans and sunflowers were planted after winter fallow [13]. In camelina seeds, GLs with long aliphatic side chains, e.g., the 9-methylsulfinylnonyl-glucosinolate and especially 10-methylsulfinyldecyl-glucosinolate (glucocamelinin), predominate accounting for apprx. 60-70% of all seed GLs [14].

The composition and concentration of GL in batches of seeds highly depend on the variety, growing area and cultivation technology [15]. For example, in the UK, in seeds of ordinary rapeseed varieties the GL content varies from 90 to 186 mmol/kg fresh weight (a moisture content of apprx. 9%), in seeds of varieties bred for low GL content from 10 to 14 mmol /kg [16]. In camelina, less GL accumulates in the seeds than in rapeseed. Thus, in 10 studied genotypes of camelina, the GL content in seeds varies from 13.2 to 36.2 mmol/kg DM and averages 24.0 mmol/kg [14]. In a more recent study of 47 camelina samples, these values were 19.6-40.3 and 30.3 mmol/kg, respectively [17].

Toxicity of glucosinolates and their effect on poultry. Goitrogenicity is the main effect of toxic products derived from GL hydrolysis and biotransformation in animals and poultry. Goitrogenic effect is a violation of the thyroid hormone synthesis and release into the bloodstream by the thyroid gland. Under more severe intoxication, the gland hypertrophies and a goiter heppens. Almost all supposed products of GL hydrolysis are involved in the goitrogenic effect, excluding (epithio)nitriles. Thiocyanate anions are direct competitors of iodine for interaction with the transmembrane protein sodium-iodine symporter (SIS) which ensures their penetration through cell membranes and binding to tyrosine residues of thyroglobulin in thyroid follicles. Oxazolidinethiones, for example goitrin derived from hydrolysis of rapeseed progoitrin inhibit the dimerization of diiodotyrosine (T2) into thyroxine (T4), the reaction of T2 with monoiodotyrosine (T1) to form triiodothyronine (T3), and the hydrolysis of thyroglobulin by the endogenous protease of the gland, followed by T3 and T4 release into the bloodstream. Although conversion of isothiocyanates to thiocyanates or oxazolidine thiones may occur, isothiocyanates are less likely to contribute to the goitrogenic effect. Together, this reduces the amount of T3 and T4 in the blood, increases the biosynthesis of thyrotropin in the anterior pituitary gland, which, in turn, activates the thyroid follicles and ultimately leads to their hypertrophy [18].

Nitriles, the hydrolysis products of GL, cause irritation of the gastrointestinal mucosa and local necrotic lesions, and exhibit hepatotoxicity and nephrotoxicity. When feeding laying hens with a 20% GL-rich rapeseed diet, alogn with a significantly lower egg production, the blood concentration of urates statistically significantly increased, and the liver enlargement occurred with reticulosis and bleeding lesions in it [19]. In turkeys fed high-GL diets for 16 weeks, fibrosis and degeneration of parenchymal cells in the central lobe of the liver occurred. From week 4 to week 12, numerous foci of necrosis appeared, and by week 16 extensive cirrhosis of the liver developed [20].

Bird kidneys and liver can quite efficiently metabolize and excret GLs and their breakdown products. In meat and even in these organs, residual amounts of these substances are practically not detected by chemical analysis. Meat quality deterioration is also not detected organoleptically even with 17-20% rapeseed in the diet [21]. However, the quatyigication of residual GL metabolites in poultry products has been carried out quite a long time ago, and the κesults may be worth reconsidering using more sensitive modern analytical methods.

The fishy smell in eggs is often due to rapeseed in the diet, especially in the more susceptible brown layers. At first, the cause was thought to be solely

rapeseed sinapin, but it was later discovered that this undesirable effect also depends on progoitrin. The fishy smell in eggs was reported to begin at 0.3 μmol/g progoitrin in a rape-free diet that corresponds to a total GL dose of 0.5 mmol/kg for brown layers and 1 μmol/g for white layers [21]. The fishy smell of eggs is due to the high content of trimethylamine (TMA) in the yolk. Bacterial fermentation of choline in the digestive tract geterats TMA that is then transferred through the bloodstream to the developing follicles in the ovary [22]. On the one hand, feeding high doses of rapeseed can lead to an excess of choline in the intestinal chyme due to the high content of sinapine in rapeseed. On the other hand, goitrin produced from rapeseed progoitrin when degraded by myrosinases or gut microflora, competitively inhibits flavin-containing monooxygenase-3 (FMO3), an enzyme that catalyzes the intestinal oxidation of TMA to the smell-less trimethylamine-N-oxide [23].

Later it was shown that the decrease in FMO3 activity is associated with a single nucleotide polymorphism (SNP) A to T at position 984 of the coding sequence of the gene for this enzyme on chromosome 8. This SNP leads to the replacement of threonine with serine at position 329 in the enzyme, The mutation can be recessive [24] or additive [25]. This mutation in the evolutionarily highly conserved sequence of FMO3 does not cause changes in the expression of the enzyme gene in genotypes that differ in the indicated single nucleotide polymorphism, the AA, TT, and AT. The fishy egg smell is most likely due to the effect of the mutation on the substrate recognition site of the enzyme [24]. Therefore, to reduce, if not eliminate this influence, laying hens with the mutation should not be used in breeding programs. In addition, it was reported that when brown laying hens of different genotypes for the specified polymorphism were fed for 4 weeks with 0, 6, 12, 18 and 24% dietary canola meal, egg yolk TMA accumulation grew linearly ($p < 0.05$) only in the homozygous mutant genotype TT, but not in AA and AT. Feeding TT laying a control diet with a higher content of synthetic choline that corresponded to the amount of sinapine in the same doses of canola meal did not increase the yolk TMA content [26]. This likely means that in canola meal, progoitrin, as an FMO3 inhibitor, makes a more significant contribution to the observed effect than sinapine, as a substrate supplier for FMO3. These data also indicate that genetic selection of laying hens can successfully address the problem of egg fishy smell at high rapeseed doses.

Subtoxic amounts of GLs in rapeseed products do not cause pronounced negative sings while relatively high doses of GLs negatively affect the efficiency of feed use and productivity performance in laying hens. In brown laying hens, 30% dietary rapeseed cake or meal, vs. no additives in control, reduced ($p < 0.01$) the egg weight, the digestibility of feed DM by 5%, crude protein by 4%, the use of gross energy by 7%, and the digestibility of all essential amino acids except for tryptophan. A 1.6% increase ($p < 0.01$) in the yolk content of monounsaturated fatty acids (MUFA) was a favorable effect [27]. The fact that these negative effects are associated specifically with rapeseed anti-nutrients, the GL and erucic acid is confirmed by another experiment. M.A. Oryschak et al. [28] assessed the efficiency of feed digestion and egg quality as affected by 7 and 14% expeller rapeseed meal in the diet vs. 30%. The four varieties used to obtain the expeller meal differ in the GL and erucic acid accumulation [28]. During 8 weeks of the experiment, the birds fed meal with the maximum GL content were inferior in digesting all main feed nutrients. The height of intestinal villi and crypt depth, egg mass and quality paramters, especially albumen height and Howe units also decreased [28]. To compare three rapeseed varieties with different seed GL contents, laying ducks were fed the meal, 10% of the diet, for 12 weeks, which significantly decreased egg weight and feed consumption [29]. The latter effect is quite common and is

traditionally explained by the sour-bitter taste of GLs and their hydrolysis products, which makes feed less attractive to animals and poultry [30]. However, the albumen height and the Hau units did not decrease compared to the control, the yolk MUFA concentration significantly decreased, which contradicted the data of another experiment [27], and the concentration of polyunsaturated fatty acids (PUFA) increased [29]. The resulting ratio of total unsaturated and saturated fatty acids remained within the control value. In all three test groups, the concentration of TMA and 5-vinyl-1,3-oxazolidine-2-thione in the yolk increased ($p < 0.01$) the more pronounced, the higher the GL content in the meal. These showed a negative effect of GL on the quality of the yolk, but not the albumen. The data obtained may indicate a certain species-specific response of birds to GLs [27-29].

In broiler chickens, moderate GL doses in diets can significantly reduce feed consumption and live weight gain without significant pathological changes due to GL toxicity, including blood concentrations of thyroid hormones and symptoms of hepatotoxicity. Interestingly, feed conversion can remain virtually unchanged [31]. The same study reveled a significant negative correlation between GL consumption and live weight gain ($r^2 = –0.74$, $p < 0.05$). It was also reported that 2-4 mmol/kg of total GL in the diet, which corresponds to approximately 20% dietary canola meal, has virtually no effect on the growth of broilers, and only doses of more than 10 mmol/kg significantly decreases average daily bodyeight gain [32]. In a recent experiment [33], 10 and 30% full-fat unmodified rapeseed in the diet led to a highly significant ($p < 0.0001$) deterioration in feed conversion, a decrease in feed consumption, a decrease in live weight and its gain compared to the control. Broilers fed 20 and 40% canola meal performed worse than controls but better than birds fed full-fat canola [33]. In another experiment [34], the addition of 20% dietary canola meal, on the contrary, did not lead to a decrease in either feed intake or growth efficiency of broilers compared to the control. The diets without rapeseed (control) and with canola meal were equal in metabolic energy, but the coarse fiber content in the control diet was noticeably less. As a result, in the cecum of chickens from the experimental group, the number of cellulolytic microorganisms increased, as well as the chyme content of short-chain fatty acids produced by these microorganisms. Short-chain fatty acids serve as energy sources for the host, which is a positive effect. However, metabolome analysis of the pancreas, liver and breast muscles of chickens showed that a diet with rapeseed, even canola, increases the risk of pancreatitis and oxidative stress in the liver. Nevertheless, the metabolic profile of the studied organs and productivity parameters indicated thay chickens can effectively counteract feed stress, at least with a moderate amount of rapeseed meal in the diet.

The differences in poultry productivity in experiments with similar amounts of rapeseed additives can be explained by both the unequal content of antinutrients, including GLs which was not determined in all cases, and the unequal availability of energy and amino acids from these additives. Indeed, in broilers, a comparative study of canola meal samples from six Canadian plants found great and significant differences in amino acid availability and metabolizable energy content [35]. In addition, differences in proteomic profiles depending on the origin of rapeseed may be important, which affects the efficiency of protein degradation by poultry enzymes and and utilization of amino acids [36].

Camelina, currently used in feed throughout the world, is much less genetically diverse than rapeseed. To our knowledge, no experiments have been conducted to evaluate the effects of purified camelina GL preparations on poultry, and the results when feeding camelina cake or meal are contradictory. This may be due to the place and conditions of the camelina cultivation and/or the oil extraction technology. Thus, 5 and 10% mechanically pressed camelina cake the

added to broiler diets from day 1 to day 37 of life linearly and significantly reduced live weight and feed consumption from day 15 to day 37 with no effect of both doses on the relative weight of the thyroid gland and the severity of symptoms of hepatotoxicity [37]. In another experiment [38], 4% camelina oil and 5 and 10% cake or full-fat seeds significantly ($p < 0.05$) reduced feed intake and live weight gain in broilers under high altitude and cold stress conditions.

Other authors reported that at a stepwise increase in the dietary camelina meal concentration from 5 to 25% during 10-37 days of life from, the feed consumption differed insignificant at 0-20% meal and decreased only at 25%. The live weight gain began to decrease significantly at doses above 15%, and the relative weight of the thyroid gland significantly increased only at 20-25% of the meal [9]. In a 42-day experiment on broilers with increasing doses of camelina cake in the diet (8-24% in 8% increments) [39], there was no significant effect of the additive on either growth rate or feed consumption. The weight of the thyroid gland also did not alter, although the blood T3 and T4 concentration grew in a dose-dependent manner. The weight of the pancreas on days 28 and 42 of life increased linearly and significantly with increasing dose of camelina. The digestibility of all main nutrients also decreased linearly ($p < 0.01$).

The advantages of camelina as a feed crop include its high content of n-3 PUFAs [40, 41]. Due to this, camelina enriches poultry eggs and meat with PUFAs and, for example, can prevent ascites in broilers at high altitudes [38]. Camelina contains from 25.9 to 36.7% α-linolenic acid ($C_{18:3}$ n-3) of the total fatty acids [41]. Feeding camelina cake (8-24%), seed (10%) or oil (2.5-6.9%) to broilers increases the content of α-linolenic acid in muscles 1.3-4.4-fold, 2.4-2.9-fold and 2.3-7.2-fold, respectively, compared to the control, and n-3 PUFAs in muscles and liver by 1.5-3.9 times [41]. Another researchers obtained similar data [42].

The results on feeding camelina cake to laying hens are contradictory. According to one report, when feeding 5, 10 and 15% camelina cake to laying hens, better egg production and the best fatty acid profile of eggs were obtained at 5%; at 15%, feed consumption significantly decreased, and shell quality deteriorated with an increase in the percentage of soft-shelled eggs [9]. In another experiment, 10 and 20% cake in the diet from week 18 to week 51 of life did not reduce either feed consumption or egg production of laying hens, and the quality of the shell, on the contrary, improved, especially with increasing age of the hens [43].

Thus, the available information on the effectiveness of camelina in poultry feeding is ambiguous, and therefore it is of interest to determine the content of GLs and, possibly, other anti-nutritional factors in camelina, as well as amino acid and fatty acid profiles.

Selection to reduce glucosinolate content. Significant accumulation of GLs, as well as erucic acid and sinapine in rapeseed seeds stimulated breeding for reduced content of these anti-nutritional factors. In 1967, an allele responsible for a significant reduction in the GL accumulation in seeds was discovered in the Polish rapeseed variety Bronowski. The introduction of this allele into varieties with a normal level of GL synthesis produced hybrids with genetically reduced GL production [44]. Since then, many low-glycoside varieties of rapeseed have been obtained, in which the amount of GLs in the seeds does not exceed 25-30 mmol/kg. Such varieties are usually called canola. However, the developed varieties turned out to be less resistant to pests, e.g., insects and wild birds, and pathogens. Since the accumulation of total GLs in seeds and leaves are traits with a high positive correlation ($r^2 = 0.79$), selection to reduce the amount of GLs in seeds led to a decrease in the GL content in leaves. Therefore, the next task was

to create low-glycoside varieties that retain effective concentrations of glycosides in the vegetative parts of the plant.

It was found that in cabbage plants, GLs synthesis occurs in three stages and involves amino acids which determine the structure of the side chain. Based on the type of these chains, GLs can be divided into three groups, These are aliphatic GLs the biosynthesis of which necessitates alanine, leucine, isoleucine, valine, methionine and its metabolites with an extended carbon chain, benzene GLs with phenylalanine and tyrosine derivatives involved, and indole GLs with tryptophan derivatives used [4, 45]. Biosynthesis of these three GL groups proceeds independently and is regulated by different sets of genes [45]. Aliphatic GLs predominate in the seeds, and benzene and indole GLs in the vegetative parts. Therefore, presumably, there could be rapeseed genotypes low in total GLs in seeds, but with a normal content of GLs in leaves. In a recent genome-wide genetic association study (GWAS) of GL biosynthesis traits in rapeseed, *BnaA03g40190D* was identified as a candidate genes responsible for this combination [46].

In addition, it turned out that the biosynthesis of GLs (including aliphatic ones) occurs predominantly in feeding tissues (leaves, walls of seed pods), and synthesized GLs are transferred to the seeds (embryos) through the phloem by GL specific transport proteins [47]. In the rhizome of *Arabidopsis thaliana* (L.) Heynh., a popular model species for studying plants biochemistry and genetics in the *Brassicaceae* family, two such proteins were found, the GTR1 and GTR2. In plants mutant for the genes of both proteins, there is no accumulation of GLs in the seeds, while the level of GLs in the leaves and pod walls is more than 10 times higher [48]. Therefore, it is possible to create rapeseed variety with reduced seed GL content by influencing the GL biosynthesis (i.e. reducing the aliphatic GL production of while maintaining indole synthesis) and expression of GL transporters [49].

Unlike rapeseed, commercial cultivation of camelina began relatively recently in world practice [2], and, as far as we know, the creation of low-glycoside commercial varieties has not yet been reported. However, there is evidence of genetic differences between varieties of camelina (*C. sativa*) and related species (*C. microcarpa*, *C. alyssum*, *C. rumelica*, *C. hispida*) in the content of some GLs [50], which indicates the fundamental basis of such selection .

Other methods for reducing the content of glucosinolates in feed products. The content of GLs and their hydrolysis products in rapeseed cakes and meals can be reduced by heat treatment at ~ 100 °C), soaking in water, treatment with aqueous solutions of alkalis or copper sulfate, solid-phase microbial fermentation, micronization, extrusion [15]. In particular, heat treatment at temperatures below 70 °C is enough to neutralize myrosinase [51]. These methods differ in their effectiveness in neutralizing GLs (from 40-45 to 90-95%). However, physical methods are very energy-intensive and therefore expensive. In addition, many of them significantly reduce the quality of the feed, in particular the solubility, ileal digestibility and absorption of crude protein and some amino acids. Rapeseed breeding is now considered the most effective strategy for reducing the content of GLs and toxic products of their biotransformation in rapeseed feed products [52, 53].

When extracting oil with solvents, subsequent heating (toasting) of the meal to desolventize it ensures fairly effective neutralization of GLs, myrosinases and associated toxins. It should be remembered that the temperature and duration of toasting have a positive effect on the efficiency of GL neutralization, but a negative effect on the feed quality of the meal. It has been shown that increasing the duration of toasting from 48 to 93 min significantly reduces the digestibility

of crude protein and most amino acids fed to pigs, and also increases the content of neutral and acidic fiber fractions in the meal [54]. Analysis of 40 samples of meal and 40 samples of cake produced in Poland from rapeseed harvest in 2003 showed that the average GL content in meal was 14.6 mmol/kg DM, in cake 17.4 mmol/kg, being within acceptable limits. It has also been shown that increasing the toasting time of both cakes and meals from 20 to 30 min reduces the coefficient of lysine availability [55]. It makes sense to toast cold (mechanically) pressed rapeseed cakes if the GL content exceeds the permissible threshold of 20 mmol/kg. For example, in a study [8], the content of total GLs in cold-pressed cake was 9.9±0.7 mmol/kg. Such cakes can be used in feed without pre-treatment, as well as for the production of protein concentrates, and during their production the GL content decreases even more.

Fermentation is among the most effective methods for reducing the GL content in rapeseed products, which maintain the amount and availability of amino acids and increase the metabolic energy content. In fermentation, specially selected compositions of microorganisms are usually used. Recently, solid-phase fermentation technology for rapeseed meal has been developed using strains of *Lactobacillus acidophilus*, *Bacillus subtilis* and *Saccharomyces cerevisiae*. It was reported that the amount of metabolizable energy increases from 7.44 MJ/kg in unfermented meal to 8.51 MJ/kg in fermented meal. Ileal digestibility (availability) of alanine, valine, isoleucine, leucine, tyrosine, lysine, arginine and phenylalanine increased, and the content of aspartic and glutamic acids, histidine, threonine, serine, proline, glycine, methionine and cystine remained at the level of unfermented meal [56]. Fermented meal not only significantly ($p < 0.05$) increased feed consumption and live weight gain in broilers compared to unfermented meal, but also significantly reduced oxidative stress. It was evidenced by increased blood concentrations of superoxide dismutase and total antioxidant capacity and decreased malondialdehyde concentration [57]. All this indirectly indicates the effectiveness of neutralization of GLs during the fermentation process. Fermentation of rapeseed meal using *Bacillus subtilis* and *Aspergillus niger* significantly reduced the GL content in it [58]. When feeding such meal to laying hens instead of 33, 66 and 100% soybean meal, significantly ($p < 0.05$) higher rates of egg production and egg weight were noted than when adding unfermented rapeseed meal. However, when soybean meal was completely replaced with both types of rapeseed meal, these parameters significantly decreased compared to the control [58].

It is also effective to simultaneously add exogenous enzymes β-glucanase, xylanase, pectinase, and cellulase to diets, which increase the digestibility of coarse fiber in rapeseed meal. It was reported [59] that 20% fermented canola meal combined with a multi-enzyme preparation containing, among others, the above enzymes, improves ($p < 0.05$) productivity, antioxidant status and immune status of chickens, i.e., bursa Fabricius weight and antibody titer against Newcastle disease, compared to groups fed 20% unfermented or fermented meal, or 20% unfermented meal with the same enzyme preparation.

For camelina cakes and meals with a high GL content, their decontamination using the desciribed methods is still quite relevant. Recently, micronization of full-fat camelina grains was shown to be effective in broiler chickens [60, 61]. However, since camelina has become widely used in feeding relatively recently, there are still few such reports, and the problem of neutralizing camelina GL requires further study. At the present stage in practical feed production and feeding, one can rely on the results obtained earlier in studies on rapeseed (with the necessary adjustments).

Recommended doses of rapeseed and camelina products in poultry diets. According to M.K. Tripathi and A.S. Mishra [15], the maximum permissible

level of GL in poultry diets can be considered 5-6 mmol/kg. For laying hens, low-glycoside varieties of rapeseed (00, canola), up to 10% of the diet, are recommended with virtually no damage to health and productivity, for broilers up to 20%. Additives from cakes and meals of conventional varieties of rapeseed should make up no more than 10-15% of the diet for broilers and 7-8% for laying hens. Preliminary toasting of these products is mandatory. When rapeseed products are used as the main source of protein in poultry diets, they can be supplemented with alternative protein sources, such as sunflower meal [62]. This also reduces the cost of the diet and the final GL content.

The amount of camelina products in the diets of broiler and laying hens is determined by the GL content and can usually be up to 5-10% [40]. The same amounts of camelina supplementation are recommended for growing meat quails [63]. There is every reason to believe that selection of camelina to reduce the GL content in seeds will open up broader prospects for the use of this crop in poultry feeding.

Finally, it is worth noting that in recent years, the beneficial effects of moderate GL doses on human have been repeatedly reported. Population studies have found that consuming a significant amount of cabbage crops reduces the risk of cancer and cardiovascular diseases. It is believed that isothiocyanates (hydrolysis products of GLs) prevent carcinogenesis, tumor growth and metastases, and also exhibit anti-inflammatory and antioxidant properties [64, 65]. Theretore, low feed doses of GLs can have similar positive effects in poultry. In particular, it was reported that allyl isothiocyanate (500 and 1000 g/t) added to the diet of broilers infested with *Eimeria maxima* improved the morphofunctional state of the intestine [33].

So, data on the toxicity and antinutritional properties of glucosinolates (GLs) and their hydrolysis products show that their high doses cause hypertrophy of the thyroid gland, hepato- and nephrotoxicity. When the dose of GLs is reduced, the function of the digestive system, feed consumption, digestibility and utilization of nutrients decrease without pronounced toxic effects. These anti-nutritional properties adversely affect the growth of young birds, decrease egg production and quality in adults and can cause fishy smell of eggs. Rapeseed and camelina and their products obtained during oil extraction contain GLs, but are valuable sources of protein necessary for feeding animals and poultry. The ability to use these products is especially important for regions where climatic conditions do not allow effective cultivation of soybeans, but more unpretentious rapeseed and camelina can be grown. To reduce the GL content in such feed additives, various methods have been developed (micronization or extrusion, microbial fermentation, in particular solid-phase). The most effective approach seems to be plant breeding to reduce the content of GLs and other antinutrients (erucic acid, sinapine). With the introduction of low-glycoside canola varieties, the problem of antinutrients in rapeseed has become less acute. However, the study of this species remains relevant for improving its quality as a fodder crop and clarifying regulations for use. In addition, these data partially compensate for the lack of such information on camelina.

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