KEMIDA.

NUTRITIONALLY ENHANCED FEED GRAINS - CREATING AND SHARING VALUE

HRANIDBENO POBOLJŠANJE KRMNOG ZRNJA - VRIJEDNOST STVARANJA I SUDJELOVANJA

P. Hooper, E. Topitschnig

Scientific review - Pregledno znanstveni članak UDC: 636.085.1. Received - Primljeno: 15 june - lipanj 1999.

SUMMARY

Improvements in grain quality contributing to the most important components of the animal diet including energy density and availability, amino acid composition and nitrogen availability, and phosphate availability are all being actively addressed in crop research (Table 11). The development of transgenic approaches has the power to greatly increase the rate and magnitude of the grain composition alterations. However, regulatory and consumer reaction is an important modifier of die rate at which these products can be introduced onto the market. Significant advances continue to be made through relying on dependable plant breeding approaches to exploit observed genotypic variation in traits of interest as well as mutants affecting those traits. A great number of additional opportunities exist in using plant breeding approaches to remove or reduce antinutritional factors in feed ingredients.

Grain with improvements in oil content and improvements in amino acid composition will very likely be available on a large scale in the next few years for animal feeding use along with grain possessing a highly available phosphorus content. In order to be successful, however, it is imperative that the grain improvements have neglible effect on crop yield. The improved traits must also either individually or collectively provide sufficient increased value to make up for the cost of preserving the identity of the grain, and yet provide sufficient financial incentive for end users and grain handlers to source the grain. Overall, it is opinion that the increased value of the improved feed ingredients will allow these products to reach the feeder at an economically viable cost.

INTRODUCTION

Grains are the major staples and the primary supplements of feeds for monogastric animals as well as food for humans. The primary emphasis in grain crop research is improvement of productivity.

However, there is also considerable importance and opportunity in improving crop quality for end users. Such improvements increase value per unit area of crop production in addition to volume. The

Dr. Peter Hooper, Pioneer Hi-Bred, France; Ing. Ernst Topitschnig, Pioneer Saaten GmbH, Austria.

opportunity for quality improvement has been recently enhanced by the development of gene transfer technologies and better analytical tools. Gene transfer technologies allow alterations in grain composition to be made more rapidly and with potentially greater magnitude and precision than was previously possible. The analytical tools allow for the accurate high-throughput analysis of grain composition that permits evaluating a grain trait in a breeding program as well as quantifying its value in the marketplace.

There are several good reviews available covering the spectrum of forage and grain improvement research efforts (Altenbach and Townsend, 1995; Halpin et al., 1995; Nutritional Quality of Cereal Grains, 1987; Cereal Grain Protein Improvement, 1984; Bright and Shewry, 1983; Seed Proteins, 1983; Anderson, 1997). This paper focuses on feed improvements particularly beneficial for monogastric animals. The areas of application include energy availability, protein quality/amino acid composition, and phosphorus availability.

ENERGY AVAILABILITY

Cereals are central to monogastric diets. They provide the vast majority of the energy and carbon required for animal growth. Therefore, maximizing the energy availability of the cereal grain is arguably the most important consideration in improving its overall value. Likewise, getting the most energy from supplements such as oilseed meal, that are added in significant volume to diets is an important consideration as well. In cereals we are only beginning to understand what characteristics at the molecular level impact the metabolisable energy of the cereal ration. Clearly, general factors such as starch quantity and availability, oil quantity, and presence of poorly digested components like fiber play major roles.

A considerable amount of work in the area of improving energy density of cereal grain has been focused on elevating the oil content of corn. Selection work at the University of Illinois over many years demonstrated that oil levels could be

increased several fold as a percentage of grain weight (Dudley et al, 1984). Oil quantity as a trait was shown to be highly heritable. However, lines possessing in excess of 7% oil displayed a yield disadvantage compared to normal lines containing 3.5-4.5% oil. DuPont Optimun Grain has recently utilized these high oil lines in a gain production system that yields gain with 6-8% oil on a dry weight basis (Araba, 1996). A primary advantage of this "top cross" system is that it permits oil levels above 6% to be obtained without serious negative impact on grain yield. However the value of such high levels of unsaturated oils in monogastric feeding systems needs further investigation.

The two main nutritional components of corn seed are the endosperm (82% on a weight basis) and embryo or germ (10%) (Glover, 1987) (Figure 1). The endosperm is composed primarily of starch and storage proteins with a poor composition of essential amino acids such as lysine. The germ possesses the vast majority of the oil and higher quality protein. The breeding selection work to develop done the high oil lines has resulted in increased germ size in proportion to endosperm and therefore a higher oil percentage. This change in the grain structure has the added effect of increasing the total protein and the quantity of essential amino acids (Table 1). Grain with 6% oil on a dry basis is described as having increases of 26°% in crude protein, 14% in lysine, 40% in tryptophan, 33% in methionine and 30% in threonine. The fatty acid profile of the high oil corn also differs from that of normal corn by having approximately 5% more monounsaturated oleic acid and a corresponding decrease in doubly-unsaturated linoleic acid (Table 2). The effect is to somewhat reduce the iodine value or degree of unsaturation of the oil though it still remains unsaturated and needs careful use in feeding. The increase in oil on a dry weight basis is offset by a decrease in starch, the net effect, though, is an increase in the amount of available energy due to the energy density of lipid being higher than carbohydrate. Feeding studies of conventional adult cockerels demonstrated a net increase in metabolisable energy (ME) between 4% and 6% depending upon the quantity of oil present (Table 3).

Figure 1. Dent Slika 1. Zrno kukuruza

DENT - UDUBINA Floury endosperm. More "open" in structure yet opaque in appearance. Crown - Kruna Dent corn about equal BRAN - PERIKARP proportions of horny to Vitreous endosperm. floury starch". called horneous, corneous or Brašnasti endosperm hard endosperm. Produced Otvoreniji" u strukturi ali grits in dry milling. Tightly nepropustan compacted and translucent. izgledom. Udubina zrna ima po prilici More of this starch in mature, jednake omjere rožnatog high test weight kernels. do brašnastog škroba. Staklasti endosperm. Naziva se i rožnati ili tvrdi endosperm KLICA Proizvodi šrot pri suhom mljevenju. Čvrsto zbijen i prividan. Više ove škrobi u zrelim Pericarp (Bran) - Perikarp košticama visoke težine. Germ scutellum and embry-Hilum or abscission laver. Also onic axis. Germ will be called blacklayer. Caused by bigger in HOC at the collapse and compression of expense of starch. For each several lavers of cells at physio-1% increase in oil, expect logical maturity. Cool weather 1.3% decrease in starch. can cause premature BL. Scutellum klice i embrion-Hilum ili sloj apscisija/odreza. ska os. Klica će biti veća u Zvan i crni sloj. Nastao slomom HOC na račun škroba. Za ili kompresijom nekoliko slojeva svakih 1% povećanja ulja stanica u fiziološkom dozrijevaočekivani pad škroba iznosi nju. Hladno vrijeme može prouz-1.3%. ročiti prerani crni sloj. TIP CAP - VRH KAPICE

Corn is normally grown in a uniform grain production field containing a single hybrid. Pioneer Hi-Bred International, Inc has an oil program that is developing high oil hybrids that can be grown in the traditional way. The hybrids will produce grain with 5-7% oil on a dry basis and yield competitively with normal lines. Quantities of grain sufficient for broad feeding evaluation will be available following the 1997 growing season, though there have been numerous practical feeding studies made to date with high oil corn (Table 4). The nature of the conclusions drawn from these studies are very dependent upon whether the high oil corn has been used to produce diets that are iso-energetic (and so potentially gives equal performance but at a lower feed cost) or used to replace existing corn in the

ration formulation thereby enhancing the energy density. In general, the benefits of high oil corn can be summarised as (Rand et al. 1997)

Formulated into balanced feeds at higher energy plateaux, improves growth rates and especially feed conversion in broilers and turkeys.

For iso-energetic diets, allows the reduction of dietary supplemental fats (the quality of which can be questionable and difficult to control) and ration inclusions of cheaper raw materials such as wheat/rice byproducts without compromising the nutritional value of the feed.

Enhanced feed production through reduction feedmill dust particles and improvement in pellet quality.

Table 1. Analytical Profiles of High Oil Corn versus normal corn

Tablica 1. Analitički profili zrna kukuruza bogatog uljem prema
normalnom zrnu kukuruza

Nutrient - Hranjivo	Normal corn Normalno zrno kukuruza 100% DM - ST	High Oil Corn Zrno kuk. s visokim postotkom ulja 100% DM - ST	Increase Povećanje
Ether extract - Sirova mast	4.03	6.12	52%
Protein - Bjelančevina	8.72	11.00	26%
Ash - Pepeo	1.34	1.46	distant
Alanine	0.60	0.84	
Arginine	0.41	0.51	
Aspartic Acid	0.57	0.72	
Cystine	0.21	0.24	15-1
Glutamic Acid	1.45	2.02	HEAT.
Glycine	0.33	0.40	
Histidine	0.24	0.30	
Isoleucine	0.28	0.36	
Leucine	0.97	1.35	
Lysine	0.28	0.32	14%
Methionine	0.18	0.24	38%
Phenylalanine	0.40	0.52	
Threonine	0.30	0.39	30%
Tryptophan	0.057	0.08	40%
Tyrosine	0.26	0.28	
Valine	0.41	0.52	

Tablica 2. Profili masnih kiselina Table 2. Fatty-acid profiles (%)

Analyte	Normal corn Normalno zrno kukuruza	High oil corn - Zrno kukuruza s visokim postotkom ulja
C 16:0 (palmitic)	11.5	10.8
C 18:0 (stearic)	2.2	2.3
C 18: (oleic)	35.5	41.2
C 18:2 (linoleic)	48.0	43.6
C 18:3 (linolenic)	1.3	0.9
Total saturates Ukupno zasićene	13.7	13.1
Total unsaturates Ukupno nezasićene	86.0	88.0

Tablica 3. ME vrijednosti MJ/Kg/ST Table 3. ME values, MJ/Kg/DM

Oil content (% dry matter) Sadržaj ulja (% suhe tvari)	ME	Improvement Poboljšanje %
4.3	16.3	111111111111111111111111111111111111111
6.6	16.9	3.7
9.5	17.3	6.1

Tablica 4. Glavne prednosti zrna kukuruza bogatog uljem Table 4. Mayor Benefits of High Oil Corn

- Formulated into balanced feeds at higher energy plateaus, improves growth rates and especially feed conversion
 in broilers and turkeys. Umiješano i izbalansirano krmivo na višim razinama energije poboljšava stope rasta a
 osobito konverziju hrane kod brojlera i purana.
- 2. For Iso-energetic diets allows the reduction of dietary supplemental fats (the quality of which can be questionable and difficult to control) and ratio inclusions of cheaper raw materials such as wheat/rice by-products without compromising the nutritional value of the feed. U Iso-energetskim obrocima omogućuje smanjenje dodatnih masti u obroku (čija kakvoća može biti upitna i teška za kontroliranje) i uključivanje jeftinijih sirovina kao što su nuz-proizvodi pšenice/riže a da se ne ugrozi hranidbena vrijednost krmiva.
- 3. Enhanced feed production through reduction in feedmill dust praticles and improvement in pellet quality. Poboljšana proizvodnja krmiva smanjenjem čestica prašine u tvornici stočne hrane i poboljšanje kakvoće peleta.

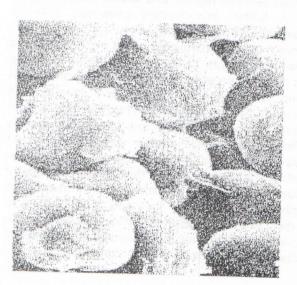
Starch, however, is the primary energy containing a component of cereal grain. There exist numerous examples of cereal mutants which possess alterations in amylase ratio or in branching (Figure 2) (Glover, 1987). These starch mutants have not been exploited in a substantial way for feed applications. The technology presently exists for potentially altering starch quantity or structure

vitreous and floury endosperm (Wassenrm et al. 1995; Halpin et al. 1995). The value of this technology to cereal improvement is being explored, particularly with regard to cereal processing (Figure 3). A single experiment using twelve commercially available hybrids showed a reduction of 88% in the cost of steam flaking between best and worst.

Figure 2. Phisical structure of the starch/protein matrix will impact the rate of starch availability Slika 2. Fizička struktura škroba/matrice bjelančevina djelovat će na stopu dostupnosti škroba.



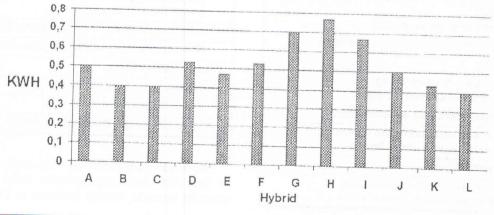
Vitreous Endosperm (Less available - Loše iskoristivo)



Floury Endosperm (More available - jače iskoristivo)

Source: Hoseney, 1986, Principles of Cereal Science and Technology

Figure 3. Steam Flaking Energy Cost Per 25 kg 1995 Pioneer Corn Hybrids Slika 3. Troškovi za energiju pare flekičenja po 25 kg hibrida zrna kukuruza Pioneer



In addition there has been increasing interest in evaluating variation in energy availability among grains produced from different corn hybrids. A study was undertaken to determine the range of energy availability from grain produced from six commercial corn hybrids (Thomas Sauber, Pioneer Hi-Bred International Inc, personal communication). Metabolisable energy was determined using individually housed pigs at 25 kg weight with total collection of faeces and urine made over a five days period (Table 5). A 6% difference was found to exist between highest and lowest values. (Figure 4). A further investigation evaluated potential differences in feed efficiency between eight different corn grain sources using pigs in an active growth stage from 25 kg to 50 kg (Table 6). Fed a diet high in corn grain (68%), a variation of 7% was found between the highest and lowest feed efficiency figures (Figure 5). The higher and lower values in each experiment were significantly different and of large enough difference to impact feed formulation. The implication of these preliminary feeding trials is that there are sufficient value differences to indicate that particular hybrid grains are preferable for feed use. Additional studies are conducted to better understand the impact of corn genetics and various environmental factors on the feeding value of corn grain.

Table 5. Metabolisable Energy Tablica 5. Metabolička energija

Hybrid Characterisation - Označavanje hibrida	
Treatments - Tretiranja	
Six commercially available hybrids Šest komercijalno dostupnih hibrida	
Diets - Obroci	
97% corn - 97% zrna kukuruza	
Vitamins + minerals - Vitamini + minerali	
Design - Plan	
10 Individually housed (25 kg) 10 Pojedinačno držanih (25 kg)	
10 pigs/treatment - 10 Svinja/tretman	
5 days collection of urine and faeces 5 dnevno skupljanje urina i fecesa	
Measurements - Mjere	
Gross energy of feed, faeces, urine Bruto energija krmiva, fecesa, urina	

Figure 4. Hybrid comparison - metabolizable energy (pigs, 25 kg)

Slika 4. Usporedba hibrid - metabolizirajuća energija (svinje, 25 kg)

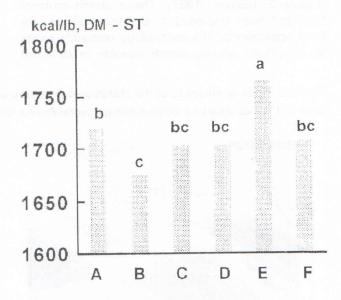


Table 6. Hybrid Feeding Value
Tablica 6. Hranidbena vrijednost hibrida

Effect of corn hybrid nibrida zrna kukuruz	l on feeding value - Djelovanje za na hranidbenu vrijednost
Treatments - Tretma	an
Seven hybrids - Sedam hibrida -	- "bin run" + "rast u posudi"/"bin run"
Diets - Obroci	
Corn/soy mixtur	e - Smjesa zrno kukuruza/soja
68% corn - 68%	zrna kukuruza
Design - Plan	
5 pens of 3 pigs 5 boksova svinj	
Initial weight 25	kg - Početna težina 25 kg
Final weight 50	kg - Završna težina 50 kg
Measurements - Mj	ere
Weight gain - P	rirast težine
Feed intake - U	nos hrane -
Feed efficiency	- Djelotvornost hrane

Although cereals provide the bulk of the energy in the diet, feed supplements used in sufficient quantity become energy sources in their own right

such as oilseed meals which we added to help meet the animal's protein quantity and quality needs. The most feasible approaches to improving the energy availability of the plant-derived supplements are by altering proportions of components with high versus low energy value or removing components that are toxic or impair digestion of the feed. Soybean meal possesses quantities of soluble oligosaccharides that are only poorly digested by monogastrics. Removal of the oligosaccharides; stachyose and raffinose, would appear to increase the overall energy content of the meal (Bajjalieh; 1996). Seed mutagenesis has been used to generate low oligosaccharide mutants with stachyose levels reduced from 4.7 to 0.1% and raffinose reduced from 0.8 to 0.2% on a dry matter basis. The reductions have been partially compensated for by an increase in sucrose from 6.5 to 9.6% (Table 7).

Figure 5. Hybrid Comparison - Feed Efficiency (Pigs, 25-50 kg)

Slika 5. Usporedba hibrid - djelotvornost krme (Svinje, 25-50 kg)

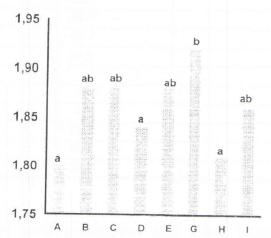


Table 7. Profiles of Regular and "Low Oligo-saccharide" Corn Types (% DM)

Tablica 7. Profili običnog tipa zrna kukuruza i tipa "niskih oligosaharida" (%ST)

	Normal Corn Normalno zrno kukuruza	Low Oligosaccharide Corn Zrno kukuruza s niskim oligosaharidima	
Stachyose	4.7	0.1	
Raffinose	0.8	0.2	
Sucrose	6.5	9.6	

Bajjalieh 1996

Protein Quality/Amino Acid Composition

The greatest degree of crop biotechnology interest and effort as it pertains to feed improvement has been focused on improved protein quality or amino acid composition. It is well known that the staples of the animal diet, the cereal grains, are low in levels of essential amino acids, particularly lysine, tryptophan, and threonine. It is therefore necessary to supplement monogastric animal diets with alternative protein/amino acid sources including oilseed meal, or synthetic amino acids. In turn, for poultry, oilseed meals are very low in additional essential amino acid, methionine, requiring supplementation from synthetic sources.

The goals of the breeding work are to improve the amino acid composition of the cereals or oilseeds to reduce the amount of supplementation required to meet the animal's essential amino acid needs and to improve the overall nitrogen utilization. The overall goal of the effort is to obtain enough accumulation of a novel storage protein with superior amino acid composition so that the overall amino acid profile of the seed is improved. The approaches that have been taken include the alteration of levels of existing storage proteins, the introduction of novel storage protein, and the deregulation of essential amino acid biosynthetic pathways to obtain accumulation of free amino acids. Several good reviews covering protein quality improvement prior to 1988 are available 1990; Osborn, 1988; Cereal Grain Improvement, 1984; Bright and Shewry, 1983; and Monti and Grillo, 1983). A review by Altenbach and Townsend (1995) covers more contemporary research.

ALTERING PROTEIN RATIOS

The earliest and most pursued approach to improving cereal protein quality/amino acid composition has been through attempts to alter ratios of endogenous seed protein fractions. With the exceptions of oats and rice, prolamines make up the major fraction of storage proteins in cereal seed endosperm. The prolamines are extremely low in lysine and have lower than average amounts of threonine, and in some cereals, tryptophan. The poor amino acid composition of prolamines is the

cause of the overall poor protein quality of cereal grain. Several spontaneous or induced mutants have been identified which reduce the prolamine fraction and increase the levels of proteins with superior amino acid balance (Bright end Shewry; 1983). Among the best known of these is the opaque 2 mutant of corn (Mertz et al., 1964). The prolamine protein fraction is substantially decreased in this mutant. Grain of opaque 2 corn has lysine levels as high as 90% above normal

(Mahan 1973) (Table 8). Both protein-bound and free lysine levels are increased (Misra et al., 1975). In general, however, the low prolamine mutants are accompanied by reduced carbohydrate content and smaller seed size (Glover, 1992). Additionally, the soft kernels of opaque 2 corn grain have increased susceptibility to kernel breakage and to pests and diseases during storage. This is a typical example of how gains in one nutritionally important area can lead to problems being developed in others.

Table 8. Composition of normal and Opaque-2 corn Tablica 8. Sastav normalnog i Opaque-2 zrna kukuruza

	Normal corn, % dry matter Normalno zrno kukuruza, % suha tvar	Opaque-2, % dry matter Opaque-2, % suha tvar
Amino acids - Amino kiseline		
Lysine	0.28	0.53
Threonine	0.37	0.41
Methionine	0.17	0.17
Cystine	0.16	0.22
Isoleucine	0.36	0.40
Leucine	1.26	1.06
Tryptophan	0.10	0.16
Proximate analysis - Neposredna analiza		
Crude protein - Sirova bjelančevina	10.44	12.6
Ether extract - SIrova mast	4.4	5.4
Fiber - Vlaknina	2.26	3.20
Ash - Pepeo	1.34	1.82
Carotene - Karotin, mg/kg	4.92	2.16

Cromwell et al.

NOVEL STORAGE PROTEINS

There has been substantial interest and activity in the past 5 years in developing and applying the technology for introducing and obtaining accumulation of novel or foreign storage proteins in plant seeds. The majority of the nitrogen and amino acids in the seed is bound in storage proteins. One of the main motivations for research activity in this area is that the efforts involve working with single genes that encode individual storage proteins instead of with multigene systems. Other reasons are that a number of protein candidates with

valuable amino acid compositions have been identified and there has been significant progress in development of gene transfer technologies for introducing genes.

Most of the transgenic storage protein work in the past 10 years has been aimed at improving the methionine content of legumes. The work has been carried out either directly with legumes or with model species that are much easier to transform. The proteins of interest for these studies are the ones that possess sufficiently high levels of methionine. The target proteins include native proteins that have been isolated from various plant

species and characterized and native proteins that have had their amino acid sequences modified to include more methionine residues. Examples of normal ones that have been mostly studies include proteins from corn, sunflower, rice, brazil nut, and soybean (Altenbach and Townsend, 1995). These proteins have methionine contents ranging from 10 to 23% on a mole percent basis.

Brazil nut albumin has fared well when introduced into other plant material. For example, a gene construct of the Brazil nut protein coding sequence was transformed into a soybean. The Brazil nut protein accumulated to levels as high as 20% of the total soluble protein of the meal, and seed methionine content increased as much as 50% over normal levels. This level of increase is significant in terms of reducing the need for supplementation of corn/soybean poultry diet with synthetic methionine. As promising is this results is that the Brazil nut protein will not be used for developing high methionine commercial plant varieties. Through evaluation of the product showed the protein to have significant allergenic properties. These allergenic properties were transferred to the target transgenic plants restricting their further development (Nordlee et al., 1996).

Overall, the technology developments for legumes suggest that there is great promise for significant improvement of protein quality through transgenic approaches utilizing storage protein genes. This approach is also being used for cereal improvement. In cereals the main target is improvement of lysine levels for feed and food uses.

There is also interest in increasing methionine content corn. Although methionine is not generally considered limited corn, increased methionine levels would have value in overcoming methionine shortages when corn/soybean diets are used for poultry feed. Attention has been given to increasing the quantity of endogenous high methionine protein in corn.

The effort to alter storage protein composition of seeds by transgenic approaches is proceeding as quickly as technology develops. As gene transfer protocols are developed for individual crop

species there soon follows a successful attempt to get a novel storage protein expressed in the seed to alter amino acid composition or other seed properties. It can be expected that the more valuable of these grain improvements will be available for feed in the next 10 years.

ACCUMULATION OF FREE AMINO ACIDS

An alternative approach to altering the storage protein contribution to the amino acid profile is to increase the levels of desired free amino acids in the seed. Typically the reserve any free amino acid is less than 5% of the protein-bound amount. The reserve amounts of free amino acids in general are tightly regulated. The amino acid biosynthetic pathways are controlled at the enzyme level by the concentration of the amino acid products of the pathways.

Early work in this area used cell culture selection approaches to identify mutants with deregulated biosynthetic pathways (Bright and Shewry, 1983).

This technique was used to identify mutants of corn that overproduced free threonine (Hibberd and Green, 1982). Corn cell lines were identified which were resistant to greater than normal levels of lysine plus threonine and produced increased quantities of threonine. Plants were generated and shown to have total seed threonine levels greater than 50% over normal seeds on a dry weight basis. The same approach was used with corn and the tryptophan analogue e 5-methyltryptophan (5MT). Corn cell lines were identified that were resistant to several fold greater levels of 5MT overproduced free tryptophan (Hibberd et al., 1986). Plants obtained from the cell cultures produced seeds with up to two fold increases in tryptophan. The levels obtained would be sufficient to meet the tryptophan needs for corn fed pigs or poultry without any supplementation.

Overall this approach to improving the amino acid composition of grain looks very promising in that substantial increases in free amino acids can be obtained, the amino acid is highly available

since it is not protein bound, the high amino acid trait is conferred by a single dominant gene, and the approach is applicable to many crop species. Significant further work is needed to transfer some of this germplasm found within naturally occurring mutants into the elite germplasm lines of current leader hybrids.

Phosphorus Availability

On the volume and cost basis phosphate is one of the most important feed additives in the diet of monogastric animals. Most feed grains have sufficient quantities of phosphorus to meet the animal's needs, however, it is not available. Most of it is present us a covalent component of phytate. Phytate accounts for the majority of the total phosphorus in cereal and over 80% of the total phosphorus in most legumes (Lolas and Markakis, 1975). The values for total phytate phosphorus content in corn and soybean are 60% and 50% respectively (Reddy et al., 1982). The phytate is poorly digested by simple-stomac animals resulting in low availability of the phosphate and contributing to phosphate waste in the environment. To make up for the poor availability, phosphate is added to the diet to meet the growing animal's needs. Phytate is also implicated in antinutritional activities in the grain. It appears to bind to proteins decreasing their digestibility (O'Dell and De Boland, 1976). It also complies with divalent minerals and possibly amino acids decreasing their availability in feed (Reddy et al., 1982). Efforts to decrease phytate content and increase free phosphate levels would decrease the need for feed supplementation

and use of phytase as en additive, reduce waste, and eliminate possible antinutritional activities.

Table 9. Composition of phosphorus from "Normal" and Low Phytate Corn

Tablica 9. Sastav fosfora "normalnog" zrna kukuruza i zrna kukuruza s niskim fitatom

	Normal corn Normalno zrno kukuruza	Low phytate Niski fitati
Total phosphorus (g/Kg/DM) Ukupan fosfor (g/kg/ST)	3.8	3.9
Phytate P (g/Kg/DM) Fitinski P (g/kg/ST)	3.2	1.4
Organic P (g/Kg/DM) Organski P (g/kg/ST)	0.45	0.5
Inorganic P (g/Kg/DM) Anorganski P (g/kg/ST)	0.15	2.0

Mutation approaches have been applied to disrupting steps in phytate biosynthesis. Corn mutants witty substantially reduced phytate content in the kernel have been identified (Gerbasi et al., 1993; Raboy and Gerbasi, 1996). The mutations result in a molar equivalent increase in inorganic phosphate (Table 9). Reductions in phytate as high as 95% have been obtained (Raboy, USDA ARS, personal communication). However, the most significant reductions are accompanied by seed problems. Phytate germination or viability reductions to approximately 1/3 the normal level appear possible without these negative effects. Preliminary feeding studies with poultry (Table 10) show the phosphorus to be more available to the animal, with less phosphorus excreted (40% less).

Table 10. Preliminary broiler feeding study using low phitate corn
Tablica 10. Preliminarno istraživanje hranidbe brojlera upotrebom zrna kukuruza niskog fitata

	Normal Corn Normalno zrno kukuruza	Low Phytate Niski fitati
Phosphorus availability (% of monosodium phosphate control) Dostupnost fosfora (%kontrola mononatrijevog fosfata)	37.1	98.6
Faecal phosphorus concentration (g/kg/DM) Koncentracija fekalnog fosfora (g/kg/ST)	3.1	1.9

Table 11. Monogastric Grain Characteristics and Value Tablica 11. Monogastrične značajke i vrijednost zrna

Trait - Obilježje	#2 Yelow #2 Žuto	K	Concept corn oncept zrna kukuruza	Rezult of change - Rezultat promjene
1. Oil - Ulje	3.5%		6%	Increased Energy Density Povećana gustoća energije
Energy availability Dostupnost energije	88%		91%	Increased useful energy density Povećana gustoća korisne energije
3. Lysine Lizin	28%		0.60%	Increased protein value Povećana vrijednost bjelančevina
4. Threonine Teonin	.40%		0.50%	Increased protein value Povećana vrijednost bjelančevina
5. Tryptophan Triptofan	.90%		0.14%	Increased protein value Povećana vrijednost bjelančevina
6. Methionine Metionin	.19%		0.36%	Increased protein value Povećana vrijednost bjelančevina
7. Phytate phosphorus Fitinski fosfor	80%	75% Decrease in phitate 75% Smanjenje fitata		Increased useful phosphorus + decreased phosphorus excretion - Povećan korisni fosfor + smanjeno izlučivanje fosfora
Nitrogen availability Dostupnost dušika		50% decrease in zein storage proteins, 6% total crude protein - 50% smanjenje pohrane zeina, 6% ukupne sirove bjelančevine		excretion
Reduced mycotoxins Reducirani mikotoksini		Mycotoxin free - Slobodni mikotoksini		Increased feed intake, animal performance & food safety - Povećano uzimanje hrane, performanca životinja i sigurnost hrane

REFERENCES

- Altenbach, S. B., J. A. Townsend (1995): In Biotechnology: in Animal Feeds and Animal Feeding, (R. John Wallace and Andrew Chesson, Eds.), Pp.71-92. Jointly published by: VCH Verlagsgesellschaft mbH, Weinheim (FRG) and VCH Publishers Inc., New York, NY.
- Altenbach, S. B., C. C. Kuo, L. C. Staraci, K. W. Pearson, C. Wainwright, A. Georgescu, J. Townsend (1992): Plant Mol: Biol, 18, 235-245.
- 3. Altenbach, S. B., K. W. Pearson, G. Meeker, L. C. Staraci, S. S. M. Sun (1989): Plant Molecular Biology., 13, 513-522.
- 4. Anderson, P. C. (1997): Crop Biotechnology for Feed Improvement (in-press).
- Anthony, J., W. Brown, D. Buhr, G. Ronhovdem, D. Genovesi, T. Lane, R. Yingling, K. Aves, M. Rosato, P. Anderson. (1996): In Third Workshop on Sulfur Metabolism, Newcastle, UK in press.
- 6. Araba, M. (1996): Annual Poultry Nutrition Conference. Carolina Feed Industry Association.

- Bagga, S., D. Sutton; J. D. Kemp, C. Sengupta-Gopalan (1992): Plant Molecular Biology, 19, 951-958.
- 8. Bajjalieh, N. L. (1996): Feedstuffs 68. 1-26.
- Beachy, R. N., Z. L. Chen, R. B. Horsch, S. G. Rogers, N. J. Hoffman, R. T. Fraley (1985): EMBO Journal, 4, 3047-3053.
- Blechl, A. E., O. D. Anderson (1996): Nature Biotechnology, 14, 875-879.
- 11. Bliss, F. A. (1990): Hort Science, 25, 1517-1520.
- 12. Bright, S. W. J., P. R. Shewry (1983): CRC Critical Reviews in Plant Sciences, 1, 49-93.
- 13. Burgoon, K. G., J. A. Hansen, D. A. Knabe, A. J. Bockholt (1992): Journal of Animal Science, 70, 811-817.
- 14. Cereal Grain Protein Improvement (1984). Viena, Austria, International Atomic Energy Agency.
- 15. Cranwell, G. L. et al. (1967): J. Anim. Sci 26:1325.
- Dudley, J. W, R. J. Lambert, D. E. Alexander (1974): In Seventy Generations of Selection for Oil and Protein in Maize, (J. W. Dudley, Ed.), pp. 181-211, Madison, Wisconsin, CSSA.

Krmiva 42 (2000), Zagreb, 4; 193-205

- 17. Falco, S. C., T. Guida, M. Locke, J. Mauvais, C. Sanders, R. T. Ward, P. Webber (1995): Biotechnology, 13, 577-582.
- Gerbasi, P., K. Young, V. Raboy (1993): In 35th Annual Maize Genetics Conference Abstracts, St. Charles, Illinois.
- Gevers, H. O., J. K. Lake (1992): In Quality Protein Maize, (Edwin T. Mertz, Ed.), pp.49-78. St. Paul, Minnesota, American Association of Cereal Chemists.
- Glassman, K. F. (1992): In Biosynthesis and Molecular Regulation of Amino Acids in Plants, (Bijay K. Singh, Hector E: Flores and Jack C. Shannon, Eds.), pp. 217-228. Rockville, Maryland, American Society of Plant Physiologists.
- Glover, D. V. (1992): In Quality Protein Maize (E.T. Mertz, Ed.), pp. 9-48. St, Paul, MN, American Association of Cereal Chemists.
- Glover, D. V., E. T. Mertz (1987): In Nutritional Quality of Cereal Grains: Genetic and Agronomic Improvement, (R.A. Olson and K.J. Frey, Eds.), pp. 183-336. Madison, Wisconsin, American Society of Agronomy.
- 23. Halpin, C., G. A. Foxon, P. A. Fentem (1995): In Biotechnology in Animal Feeds and Animal Feeding, (R. John Wallace and Andrew Chesson, Eds.), pp. 279-293. Jointly published by: VCH Verlagsgesellschaft mbH, Weinheim (FRG) and VCH Publishers Inc. New York, NY.
- 24. Hayashimoto, A., Z. Li, N. Murai (1990): Plant Physiology; 93, 857-863.
- Hibberd, K. A., M. Barker, P. S. Anderson (1987): United States Patent 34, 581,847.
- 26. Hibberd, K. A, C. E. Green (1982): Proceedings of the National Academy of Sciences of the United States of America, 79, 559.
- Higgins, T. J. V., D. Spencer (1991): Plant Science, 74, 89-98.
- Hoffman, L. M., D. D. Donaldson, R. Bookland, K. Rashka, E. M. Herman (1987): EMBO Journal, 6, 3213-3221.
- 29. Hoffman, L. M., D. D. Donaldson, E. M. Herman (1988): Plant Molecular Biology, 11, 717-729.
- Hovenkamp-Hermelink, J. H. M., E. Jacobsen, A. S. Ponstein, R. G. F. Visser, G. H. Vos-Scheperkeuter, E. W. Bijmolt, J. N. deVries; B. Without, W. J. Feenstra (1987): Theoretical and Applied Genetics, 75, 217-221.
- 31. Kermode, A. R., S. A. Fisher, F. Polishchuk, C. Wandelt, D. Spencer, T. J. V. Higgins (1995): Planta, 197, 501-513.

- 32. Kirihara, J. A., J. B. Petri, J. Messing (1988): Gene, 71, 359-370.
- Knabe, D. A., J. S. Sullivan, K. G. Burgoon, A. J. Bockholt (1992): In Quality Protein Maize, (Edwin T. Mertz, Ed.), pp.225-238. St. Paul, Minnesota, American Association of Cereal Chemists.
- Kortstee, A. J., A. M. S. Vermeesch, B. J. deVries, E. Jacobsen, R. G. F. Visser (1996): Plant Journal, 10, 83-90.
- 35. Lolas, G. M., P. Markakis (1975): Journal of Agricultural Food Chemistry, 23, 13-15.
- Lopes, M. A., K. Takasaki, D. E. Bostwick, T. Helentjaris, B. A. Larkins (1995): Molecular and General Genetics, 247, 603-613.
- 37. Lopes, M. A., B. A. Larkins (1995): Theoretical and Applied Genetics, 91; 274-281.
- 38. Maga, J. A. (1982): Journal of Agricultural and Food Chemistry, 30, 1-9.
- Magnavaca, R., B. A. Larkins, R. E. Schaffert, M. A. Lopes (1993): In International Crop Science I, pp. 649-653. Madison, Wisconsin, Crop Science Society of America.
- Mertz, E.T., L. S. Bates, O. E. Nelson (1964): Science, 145, 279-280.
- 41. Misra, P. S., E. T. Mertz, D. V. Glover (1975): Cereal Chemist, 52, 844-848.
- 42. Monti, L.M., S. Grillo (1983): Qualitas Plantarum Plant Foods for Human Nutrition, 32, 253-266.
- 43. Negrutiu, I., A. Cattoir-Reynaerts, I. Verbruggen, M. Jacobs (1981): Archives Internationales de Physiologie de Biochimie, 89, 188.
- 44. Nordlee, J. A., S. L. Taylor, J. A. Townsend, L. A. Thomas, R. K. Bush (1996): The New England Journal of Medicine, 334, 688-692.
- 45. Nutritional Quality of Cereal Grains: Genetic and Agronomic Improvement (1987): (R.A. Olson and K.J. Frey, Eds.). Madison, Wisconsin, American Society of Agronomy, Inc.
- 46. O'Dell, B. L., A. De Boland (1976): Journal of Agricultural Food Chemistry, 24, 804-808.
- 47. Osborn, T.C. (1988): CRC Critical Reviews in Plant Sciences, 7, 93-116.
- 48. Paez, A.V., J. L. Helm, M. S. Zuber (1969): Crop Science, 9, 251-252.
- 49. Pen, J., T. C. Verwoerd, P. A. van Paridon, R. F. Beudeker, P. J. M. van den Elzen, K. Geerse, J. D. van der Klis, H. A. J. Versteegh, A. J. J., van Ooyey, A. Hoekema (1993): Bio/Tehnology, 11, 811-814.
- 50. Raboy, V., P. Gerbasi (1996): In Subcellular Biochemistry, (B B. Biswas and S. Biswas, Eds:), Vol 26, pp. 257-285. New York, Plenum Press.

204

- Rand, N. et al, (1997): Proceedings Poultry Science Association Annual Meeting, University of Georgia, USA.
- 52. Reddy, N. R., S. K. Sathe, D. K. Salunkhe (1982): In Advances in Food Research, (C.O. Hichester, Ed.), 28; p. 1. New York, Academic Press.
- 53. Saalbach, G., V. Christov, R. Jung, I. Saalbach, R. Manteuffel, G. Kunze, K. Brambarov, K. Müntz (1995): Molecular Breeding, 1, 245-258.
- 54. Saalhach, I., D. Waddell, T. Pickardt, O. Scheider, K. Müntz (1995): Journal of Plant Phisiology, 145, 674-681.
- 55. Seed Proteins (1983): (Werner Gottschalk, Hermann P. Muller, Eds.). The Hague. The Netherlands, Martinus Nijhoff/Dr. W. Junk Publishers.
- Sengupta-Gopalan, C., N. A. Reichert, R. F. Barker,
 T. C. Hall, J. D. Kemp (1985): Proceedings of the National Academy of Science, USA, 82, 3320-3324.
- 57. Shimamoto, K., R. Terada, T. Izawa, H. Fujimoto (1989): Nature, 338, 274-276.
- Sindhu, A. S., Z. Zheng, N. Murai (1995): Plant Physiology 108 (2 Suppl.), 151.

- Stark, D. M., K. P. Timmerman, G. F. Barry, J. Preiss, G. M. Kishore (1992): Science, 258, 287-292.
- Tabe, L. M., C. M. Higgins, W. C. McNabb, T. J. V. Higgins (1993): Genetics, 90, 181-200.
- Townsend, J. A., L. A. Thomas, E. S. Kulisek, M. J. Daywalt, K. R. K. Winter, D. J Grace, W. J., Crook, H. J., Schmidt, T. C. Corbin, S. B. Altenhach (1992): In Agronomy Abstracts, p. 198, Minneapolis, Minnesota.
- 62. Utsumi, S., S. Kitagawa, T. Katsube, I. J. Kang, A. B. Gidamis, F. Takaiwa, M. Kito (1993): Plant Science, 92, 191-202.
- Villegas, E., S. K. Vasal, M. Bjarnason (1992): In Quality Protein Maize, (Edwin T. Mertz, Ed.), pp.27-48. St. Paul, Minnesota, American Association of Cereal Chemists.
- 64. Wandbelt, C. I., M. R. L. Khan, S. Craig, H. E. Schroeder, D. Spencer, T. J. V Higgins (1992): The Plant Journal, 2, 181-192.
- 65. Wasserman, B. P., C. Ham, C. Mu-Forster, R. Huang (1995): Cereal Foods World; 40, 810-817.
- Zheng, Z., K. Sumi, K. Tanaka, N. Murai (1995): Plant Physiology, 109, 777-786.

SAŽETAK

Poboljšanje kakvoće zrna, što pridonosi najvažnijim komponentama hrane životinja uključuju i gustoću i dostupnost energije, sastav amino kiselina i dostupnost dušika predmet su istraživanja usjeva: (Tablica 11). Razvoj transgenih pristupa ima sposobnost uvelike povećati brzinu i veličinu promjene sastava zrna. Međutim, reakcija regulative i potrošača važan je modifikator brzine kojom ovi proizvodi mogu doći na tržište. Do znatnog napretka i dalje dolazi oslanjanjem na pouzdane pristupe u uzgoju biljaka da bi se iskoristile primijenjene genotipske varijacije u zanimljivim varijacijama kao i mutanti koji djeluju na te značajke. Postoji veliki broj dodatnih mogućnosti u uzgoju biljaka da se odstrane ili smanje nehranjiviantinutritivni sastojci u krmi.

Zrno s poboljšanim sadržajem ulja i poboljšanim sastavom amino kiselina vrlo će vjerojatno biti dostupno na širokoj osnovi u sljedećih nekoliko godina za hranidbu životinja zajedno sa zrnom s visoko dostupnim sadržajem fosfora. Da bi se uspjelo, međutim, važno je da poboljšanje zrna ima zanemariv utjecaj na prinos usjeva. Poboljšane značajke moraju isto tako, pojedinačno ili skupno, dati dovoljno poboljšane vrijednosti da se nadoknadi trošak sačuvanja identiteta zrna i istovremeno pruži dovoljna financijska potpora krajnjim korisnicima i trgovcima zrnja.

Općenito, mišljenje je da će povećana vrijednost poboljšanih sastojaka krme omogućiti da ti proizvodi dođu do uzgajača po gospodarski održivoj cijeni.