CHAPTER 2

The Importance of Dietary Protein in Human Health: Combating Protein Deficiency in Sub-Saharan Africa through Transgenic Biofortified Sorghum

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Abstract Child malnutrition is increasing in Africa. Protein deficiency is an important cause since protein is essential for both growth and maintenance of muscle mass. Sorghum is a major staple food in Africa on account of its hardiness as a crop. However, sorghum protein is very deficient in the indispensable amino acid lysine and on cooking has poor protein digestibility. This results in sorghum having a very low Protein Digestibility Corrected Amino Acid Score (PDCAAS). The Africa Biofortified Sorghum project, a Grand Challenges in Global Heath project, is undertaking research to biofortify sorghum in terms of protein and micronutrient quality using genetic engineering. Lysine and protein digestibility have been improved by suppression of synthesis of the kafirin storage proteins. Transgenic biofortified sorghum has double the PDCAAS of conventional sorghum. This improvement should enable a young child to meet most of its protein and energy requirements from biofortified sorghum porridge. This together with the improvement in micronutrients could provide the basis of a sustainable and broadly comprehensive solution to child malnutrition in many African countries.

I. INTRODUCTION

Globally, the incidence of childhood malnutrition is declining. De Onis *et al.* (2004) reported on trends and prevalence for the years 1990–2005. His group looked at underweight and stunting data using World Health Organization (WHO) developed methodology to plot and predict trends at country levels. They found that stunting and underweight prevalences declined from 34% to 27% and 27% to 22%, respectively. However, in Africa the situation is not improving. The numbers of stunted and underweight children increased from 40 to 45 million and 25 to 31 million, respectively. Africa and subregions have extensive protein–energy malnutrition (PEM) in children under 5 years of age (FAO, 2008a, 2009). According to FAO 2009 food security statistics (FAO, 2009), the percentage of these children who are moderately and severely malnourished in the underweight category in Burkina Faso, Kenya, and South Africa, representing countries from west, east, and southern Africa, are 32%, 25%, and 15%, respectively. In the stunting category, the combined percents of children who are moderately and severely malnourished are 36%, 50%, and 39% and in the category of wasting, the combined percents of moderately and severely malnourished children are 19%, 7%, and 7%, respectively. Beyond growth retardation, under-nourished children are at risk for infectious diseases, diarrhea, and diminished mental development.

De Onis *et al.* (2004) speculate that the lack of progress in Africa may be due in part to the impact of the human immunodeficiency virus (HIV),

which causes the condition AIDS. In sub-Saharan Africa, an estimated 333,000 children below 5 years of age died in 1999 with HIV infection and an estimated 11 million were orphaned because of AIDS. The United Nations International Children's Fund (UNICEF, 2009) reported that in sub-Saharan Africa in 2007, the annual number of under-5 deaths was 4,480,000 and the life expectancy at birth was only 50 years. There are many reasons for deaths in children under 5 years of age, shortened life spans, stunting, and underweight, but major among them is an inadequate diet including protein intake.

The major staple foods in Africa are cassava (105 million tons), maize (48 million tons), yam (45 million tons), sorghum (26 million tons), plantain (24 million tons), rice (21 million tons), wheat (19 million tons), millets (18 million tons), sweet potato (12 million tons), and banana (12 million tons) (FAOSTAT, 2007). Among these, sorghum occupies a unique position on account of its hardiness as a crop, including low water usage, drought-tolerance, and resistance to water-logging (Doggett, 1988). Hence, it is a staple food of many of Africa's most food-insecure people, who live in the desert-margin, semiarid tropics, some 300 million people (ICRISAT, 2009).

The Grand Challenges in Global Health are an initiative of the Bill and Melinda Gates Foundation (BMGF). They were announced in 2003, having been developed by a team of 20 scientists and public health experts from 13 countries (Varmus *et al*., 2003). Fourteen Grand Challenges were identified in seven areas. Grand Challenge 9 (GC 9) is to ''Create a full range of optimal bioavailable nutrients in a single staple plant species'' with the aim of improving nutrition to promote health. Today in 2010, there are four GC 9 projects, all of which are supported by the BMGF. They are concerned with biofortification of major staple foods in developing countries. Biofortification can be defined as a process to increase the bioavailability and the concentration of nutrients in crops through both conventional plant breeding (White and Broadley, 2005) and recombinant DNA technology (genetic engineering) (Zimmermann and Hurrell, 2002). Polleti *et al.* (2004) in a review of the progress made in the nutritional fortification of cereals stated that effective biofortification of cereal staples can reach the poor in rural areas, has low recurrent costs, is sustainable in the long term, and in the case of genetic improvement, it only requires an up-front investment.

The GC 9 projects are Micronutrient Improved Bananas (Grand Challenges in Global Health, 2010), Biocassava Plus (2010), Golden Rice (2010), and Biosorghum (2010). The Biosorghum project differs somewhat from the other CG 9 projects in that it has aimed to improve the quality and availability of the macronutrient protein as well as the quantity and availability of micronutrients. As will be seen, protein is a uniquely important issue in sorghum, compared to other cereals. The Biosorghum

project is a consortium, the Africa Biofortified Sorghum (ABS) consortium, of some 10 organizations in Africa and the United States (Biosorghum, 2010; Zhao, 2007). It is led by the Africa Harvest Biotechnology Foundation International based in Nairobi, Kenya.

The specific aims of the Biosorghum project were to increase iron and zinc availability by 50%, to increase provitamin A levels to up to 20 mg/kg, to increase lysine content by 80–100%, to increase tryptophan and threonine by 20%, to concomitantly decrease leucine by 15%, and to improve protein digestibility from its current to approx. 60–80% (Grand Challenges in Global Health, 2010). These improvements in sorghum nutrient content and availability are in comparison with current average levels in sorghum.

This chapter sets out the role and importance of protein in human health, human protein requirements, and the measurement of food protein quality. Specifically, focus is given to the need to improve sorghum protein quality and the developments that have already taken place in this area are reviewed. Lastly, the improvements in sorghum protein quality achieved to date in the Biosorghum project are presented and on the basis of these data, the potential of biofortified sorghum to improve children's nutritional status is evaluated.

II. ROLE AND IMPORTANCE OF PROTEIN IN HUMAN HEALTH

Protein is essential for life from the beginning of gestation through old age (WHO/FAO/UNU Expert Consultation, 2007). Dietary protein recommendations are based on the requirements for indispensable amino acids, conditionally indispensable amino acids and nitrogen that is needed for syntheses of dispensable amino acids, and other critical nonprotein nitrogen-containing molecules necessary to support growth, tissue repair, and maintenance (Institute of Medicine of the National Academies, 2005). The indispensable amino acids, up to nine, cannot be synthesized by humans and therefore must be consumed in diets. With adequate precursors, the dispensable amino acids and conditionally indispensable amino acids can be synthesized by the human body. These latter amino acids are needed from dietary sources during rapid growth or during physiological stress when synthesis cannot keep up with demand. Table 2.1 shows the classification for amino acids by dispensability (Laidlaw and Kopple, 1987). Of the indispensable amino acids, lysine is present in human muscle at the highest level, at 9.78% (Wolfe and Chinkes, 2005), indicating its critical nature both in amino acid metabolism and in diets. In cereal-based diets, lysine is normally the most limiting indispensable amino acid, as cereals

TABLE 2.1 Indispensable, dispensable, and conditionally indispensable amino acids in the human diet

Indispensable	Dispensable	Conditionally indispensable	Precursors of conditionally indispensable
Histidine	Alanine	Arginine	Glutamine/glutamic acid/aspartic acid
Isoleucine	Aspartic acid	Cysteine	Methionine, serine
Leucine	Asparagine	Glutamine	Glutamic acid/ammonia
Lysine	Glutamic acid	Glycine	Serine, choline
Methionine	Serine	Proline	Glutamic acid
Phenylalanine		Tyrosine	Phenylalanine
Threonine			
Tryptophan			
Valine			

Laidlaw and Kopple (1987).

have an average lysine content of 31 mg/g protein compared to 65 mg/g in legumes and 85 mg/g in animal foods (Young *et al*., 1998).

A typical well-nourished 70-kg male contains about 11 kg of protein, with a little less than half as skeletal muscle (43%) (Lentner, 1981). Structural tissues such as skin and blood each constitute about 15% of total body protein. The metabolically active tissues such as liver and kidney (only about 10% together) contain relatively little protein. Other organs such as heart, brain, lungs, and bone account for the rest. These relative amounts change with age. For example, infants have significantly less muscle, proportionately, and more brain and visceral protein than adults. Interestingly, almost 50% of the protein within humans is composed of just four types: myosin, actin, collagen, and hemoglobin. Myosin and actin are muscle proteins and collagen is a structural protein.

In the human body, protein and other nitrogen-containing compounds are continuously broken down and contribute to an amino acid/nitrogen pool from which precursors and amino acids are reused to synthesize enzymes, hormones, lean tissue, immune function proteins, muscle mass, bone matrix, and other essential compounds (Institute of Medicine of the National Academies, 2005). Maintenance of the protein content of certain tissues and organs, such as skin, brain, heart, liver, and kidneys, is essential for survival. In chronic protein deficiency, lean tissue and muscle mass are sacrificed to provide amino acid precursors for synthesis of critical compounds, such as insulin and hemoglobin (Hoffer, 1994).

To compound the situation, when caloric intake is inadequate, amino acids are oxidized and used to synthesize glucose as an energy source for those tissues such as red blood cells that only use glucose (Guyton and Hall, 1996). If fat stores are available, fat can be used for much of the body's energy demands. However, in stunted and wasted children and adults suffering from inadequate dietary protein, calories, and other nutrients, fat stores are limited and muscle mass is tapped for amino acids to synthesize both essential protein molecules and glucose for energy. Picou *et al*. (1966) pointed out that in induced malnutrition, the proportion of body collagen can rise to 50% because of the substantial loss of noncollagen proteins, presumably muscle.

A. Importance of muscle mass

Clinical signs of protein–calorie malnutrition such as marasmus, kwashiorkor, or marasmus–kwashiorkor and various degrees of wasting, stunting, or weight loss are indicators that malnutrition is affecting muscle mass (Torun and Chew, 1994). While it is well recognized that muscle is important in the performance of the activities of daily life, Wolfe (2006) emphasizes the role of muscle in health and disease, pointing out that altered muscle metabolism plays a key role in the genesis of many common pathologic conditions. Under normal circumstances, the demands for amino acids in most organs and tissues do not vary significantly from the fasted state to the fed state because little surplus protein is accumulated in the body.

According to animal studies (Swick and Benevenga, 1977), a labile protein reserve that can be gained or lost from the body for emergencies or to account for the day-to-day variations in dietary protein intake is contained in the liver and visceral tissues. Rapid starvation or dietary protein depletion results in a loss of the reserve by as much as 40%, while skeletal muscle drops much more slowly. Thus, protein breakdown becomes the source of indispensable amino acids needed for synthesis of critical proteins necessary for body functions (Reeds *et al*., 1994) and provides amino acids as precursors for energy production.

Of the energy nutrients (protein, fat, and carbohydrate), only fat can be stored by the body in unlimited amounts. Carbohydrate is stored in limited amounts primarily in the form of glycogen in the liver and to a lesser extent in muscle where it provides a ready supply of energy for muscle movement (Guyton and Hall, 1996). Clearly, glycogen storage in muscle is limited by the amount of total muscle mass. Amino acids will be deaminated (nitrogen removed) and used as precursors to provide energy in a caloric deficit, thus the need for adequate calories to spare protein. In the course of normal events, amino acids are stored in muscle up to a limited amount to replace the amino acids lost in the previous fasting

state. Excess amino acids not needed for the body's amino acid repletion or energy demands are finally deaminated with the nitrogen from the amino acid used to synthesize urea for urinary excretion. The remaining carbon skeleton can be stored via synthesis of glycogen or fat.

Under normal situations and normal dietary intake, gains and losses of amino acids from muscles are in equilibrium. However, during physiological stress such as chronic hunger, pregnancy, burns, HIV/AIDS, cancer, starvation, and trauma, precursor amino acid demands are increased to provide for synthesis of acute-phase proteins, immune function proteins, and proteins needed for wound healing, etc. This results in rapid depletion of the reserve, which accounts for only about 1% of total body protein (Young *et al*., 1968). The protein lost during fasting is functional body protein and therefore unlike fat or carbohydrate (glycogen) stores. There is no evidence for a protein reserve that serves only as a store to meet future amino acid/nitrogen needs. Kotler *et al*. (1998) reported a strong association between the depletion of body cell mass (apparently reflecting muscle loss) and the length of survival of seriously ill patients with AIDS. Studies conducted in the Warsaw ghetto between February and July 1942 suggest that death from starvation with no complicating illness occurs when muscle protein breakdown becomes inadequate to maintain the necessary supply of precursor amino acids to make glucose (Winick, 1979). Extensive work by Keys *et al*. (1950) also concludes that the depletion of muscle mass is the cause of death in human starvation.

The relationships of muscle mass to obesity, insulin resistance, and bone health are further indications of how important it is to protect muscle mass (Wolfe, 2006). For individuals in underresourced countries, suffering from wasting-malnutrition with less than optimum muscle mass and strength, bone modeling and remodeling are at risk, since mechanical force (exerted by muscle mass and strength) on bone is essential for the processes that increase bone strength and bone mass. Changes in bone mass and muscle strength track together over the life span (Frost, 1997).

Szulc *et al*. (2005) reported that skeletal muscle mass was correlated positively with bone mineral content and bone mineral density in the Mediterranean Intensive Oxidant Study (MINOS). MINOS was a prospective study of osteoporosis and its determinants that showed that men with the least skeletal mass also had increased risks of falls due to impaired static and dynamic balance. The relative importance of muscle compared with hormonal and other nutritional effects on bone health may be argued in the MINOS study because factors such as dietary protein, insulin growth factor, and testosterone also affect bone directly, but chronically low muscle mass may provide a visible indicator of bone mass and bone density risk. Osteopenia and osteoporosis are seen as problems in elderly people. In countries with shortened life spans, bone health issues may go unnoticed.

Short-term changes in diets suggest that the main loss of protein is from the viscera (De Blaauw *et al.*, 1996). However, in chronic illness, skeletal muscle, which comprises over 40% of the protein mass of a healthy individual, becomes the largest single contributor to protein loss (Hansen *et al.*, 2000). While several factors play a role in maintaining muscle mass (exercise, hormones, and diet), clearly the importance of adequate dietary protein intake cannot be debated. Not only health, but activities of daily living, quality of life, and productivity are affected (FAO, 2009; Winick, 1979).

B. Human protein requirements

Protein requirements, or more specifically amino acid and nitrogen needs, vary depending on age, body size, gender, physiological states (including pregnancy, illnesses, and fitness), and possibly environment. Research, including nitrogen balance studies, and tracer isotope methodologies are used to determine amino acid and protein requirements for various ages, gender, gestation and lactation, and physiologic conditions (exercise, burns, and illnesses) (Institute of Medicine of the National Academies, 2005). Official amino acid/protein requirements are made for healthy persons of various ages, each trimester of pregnancy, and the initial and latter periods of lactation. Nutrition (protein) security planning for populations requires that in addition to the needs for maintaining health, consideration must be given to the prevalence and increased amino acid/protein requirements of individuals with special needs such as protein malnutrition (stunting and wasting) and illnesses.

In 2002, an expert consultation of international amino acid/protein scientists was convened by the United Nations World Health Organization, Food and Agriculture Organization, and University (WHO/FAO/UNU) to evaluate studies to-date and make recommendations for human protein intake (WHO/FAO/UNU Expert Consultation, 2007). That body had previously asked Rand *et al.* (2003) to prepare a meta-analysis of nitrogen balance studies. The meta-analysis, consisting of 235 individual subjects across 19 studies was used in the 2007 report, along with other research to make these current WHO/FAO/UNU Expert Consultation (2007) recommendations. The amino acid requirements for adults in the previous FAO/WHO/UNU Expert Consultation Report in 1985 (FAO/WHO/UNU Expert Consultation, 1985) had been taken directly from the 1973 FAO/WHO report (FAO/WHO, 1973). However, since 1985 concern had arisen regarding the previously derived values and that the values for adults were too low (WHO/FAO/UNU Expert Consultation, 2007).

Table 2.2 shows the 2007 WHO/FAO/UNU adult indispensable amino acid requirements compared to the 1985 FAO/WHO/UNU requirements. It should be noted that the levels of intake for indispensable

TABLE 2.2 Comparison of the 1985 FAO/WHO/UNU and 2002 WHO/FAO/UNU Expert Consultations[a] mean adult indispensable amino acid requirements without the coefficient of variation added

Amino Acid	2002 (mg/kg body weight/day)	2002 (mg/g protein[b])	1985 (mg/kg body weight/day)	1985 (mg/g protein)[b]
Histidine	10	15	8–12	15
Isoleucine	20	30	10	15
Leucine	39	59	14	21
Lysine	30	45	12	18
Methionine + cysteine	15	22	13	20
Methionine	10	16	—[c]	—
Cysteine	4	6	—	—
Phenylalanine + tyrosine	25	38	14	21
Threonine	15	23	7	11
Tryptophan	4	6	3.5	5
Valine	26	39	10	15
Total indispensable amino acids	*184*	*277*	*93.5*	*141*

[a] 1985, FAO/WHO/UNU Expert Consultation (1985), 2002, WHO/FAO/UNU Expert Consultation (2007).
[b] Mean nitrogen requirement of 105 mg nitrogen/kg per day (0.66 g protein/kg per day).
[c] Not given separately.

amino acids shown in the first column of the table do not take into account the variability of individual requirements. If these are taken into account, the safe levels of intake across populations for indispensable amino acids are 24% higher than shown. The 24% calculation is based on the coefficient of variation of the requirements for protein of 12%. For example, in Table 2.2 the mg indispensable amino acids/g protein is based on an intake of 0.66 g protein/kg/day, but the recommended safe level of protein for adults across genders and ages is currently 0.83 g/kg/day (WHO/FAO/UNU Expert Consultation, 2007). This safe level protein recommendation takes into account the 12% coefficient of variation so as to cover 97.5% of the population and is based on a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.0, the highest possible protein quality score since values over 1.0 are rounded down. The issue of protein quality is discussed in detail later.

Concerning the major differences in recommendations, the increases of 150% in mg/kg body weight/day adult requirement for lysine and 114% increase in requirement for threonine between the two report recommendations are of particular significance. Cereal proteins are particularly deficient in these amino acids (Young *et al.*, 1998) and as shown earlier, cereals are the major dietary staple across Africa.

TABLE 2.3 Amino acid scoring patterns mg/g protein requirements of infants, children, adolescents and adults over 18 (genders combined)[a]

Amino Acid	0.5 year	1–2 years	3–10 years	11–14 years	15–18 years	>18 years
Histidine	20	18	16	16	16	15
Isoleucine	32	31	31	30	30	30
Leucine	66	63	61	60	60	59
Lysine	57	52	48	48	47	45
Methionine + cysteine	28	26	24	23	23	22
Phenylalanine + tyrosine	52	46	41	41	40	38
Threonine	31	27	25	25	24	23
Tryptophan	8.5	7.4	6.6	6.5	6.3	6.0
Valine	43	42	40	40	40	39

[a] WHO/FAO/UNU Expert Consultation (2007).

Table 2.3 shows the amino acid scoring patterns based on the requirements for dietary indispensable amino acids for humans of different ages as determined by the 2002 WHO/FAO/UNU Expert Consultation (2007). Cysteine is paired with methionine since methionine can be used by the body to synthesize cysteine. Phenylalanine is paired with tyrosine since phenylalanine can be used by the body to make tyrosine. The progressively higher requirement for better quality protein for youths, adolescents, preadolescents, preschool children, and infants compared to adults, on account of formers' requirement for growth as well as maintenance put them at particular nutritional risk. Hence, the appropriate amino acid scoring pattern needs be used in evaluating protein quality and in the determination of PDCAAS of food proteins for people of different ages. When determining the protein adequacy of particular diets, the most at-risk consuming that diet should be taken into account.

Special populations such as pregnant and lactating women, infants, preschool children, and the elderly are at nutritional risk under the best of circumstances, but their vulnerability increases when diseases such as HIV/AIDS, or poverty, civil conflicts, and drought are superimposed. During pregnancy and lactation, both protein and energy requirements are increased. The additional safe intake for protein during pregnancy in g/day increases from 1 g/day in the first trimester to 10 g/day in the second trimester to 31 g/day in the third trimester. For example, a 50-kg adult woman, in the third trimester, will require 72.5 g of good quality protein per day (Institute of Medicine of the National Academies, 2005; WHO/FAO/UNU Expert Consultation, 2007). Pregnant teens require even more protein as they will have continued growth requirements during gestation.

III. PROTEIN QUALITY AND ITS MEASUREMENT

Dietary protein adequacy (aside from the energy/protein relationship) is a function of several protein-dependent factors: the protein content of the food, the digestibility/bioavailability of the protein/amino acids within the food, the quantity of indispensable amino acids within the protein, and the relative ratio of the amino acid content to the requirements of the person(s) or amino acid standard in question. The final protein quality score is dependent on the lowest level of bioavailable indispensable amino acid, the limiting amino acid.

Over the years, different methods have been used for the determination of protein quality for humans and production animals, with the general standard method being the Protein Efficiency Ratio (PER) (FAO/WHO Expert Consultation, 1991). PER can be defined as weight gain of test group/protein consumed by test group. However, according to the 1991 FAO/WHO Expert Consultation, a major criticism of the PER assay is its inability to credit protein used for maintenance purposes. For example, a protein source may not support growth and therefore have a PER of zero, but yet it may be adequate for maintenance purposes. The Expert Consultation proposed that PER therefore be replaced by an assay based on measures of the digestibility (bioavailability) of the protein in a food and the amino acid composition of its protein, the PDCAAS. PDCAAS predicts the biological value (BV) of a food, the effectiveness with which absorbed dietary nitrogen can be utilized (WHO/FAO/UNU Expert Consultation, 2007). In turn, PDCAAS takes into account human indispensable amino acid requirements. The FAO/WHO Expert Consultation (1991) proposed PDCAAS as a means of assessing protein quality of both dietary mixtures and individual food proteins. Shortly afterwards, PDCAAS was adopted by the United States Food and Drug Administration for its 1993 nutrition labeling regulations (Henley and Kuster, 1994). PDCAAS was further endorsed by the WHO/FAO/UNU Expert Consultation (2007) and in South Africa, for example, has been adopted for food labeling legislation (South African Department of Health, 2002).

PDCAAS is calculated as true protein digestibility (TD) × Amino Acid Score (AAS) (WHO/FAO/UNU Expert Consultation, 2007).

$$\text{True protein } (N) \text{ digestibility } (\%) = \frac{I - (F - F_k) \times 100}{I}$$

where I is the nitrogen intake, F is the fecal nitrogen loss on the test diet, and F_k is the fecal nitrogen loss on a protein-free diet.

TD is determined by rat bioassay (AOAC International, 2000), although as will be seen the rat may not be an appropriate model to estimate the protein digestibility of sorghum in humans.

AAS = mg of limiting amino acid in 1 g test protein/mg amino acid in required pattern (WHO/FAO/UNU Expert Consultation, 2007).

As an example, Table 2.4 shows PDCAAS calculations for sorghum for different age groups based on their indispensable amino acid requirements according to the WHO/FAO/UNU Expert Consultation (2007). Since the AAS shows that lysine is the most limiting amino acid in sorghum, in this case PDCAAS is based on lysine. A TD value of 74% (Daniel *et al.*, 1966; Hopkins, 1981) obtained from a child feeding intervention study was applied. However, as will be seen below, this figure is probably an overestimate for sorghum. Irrespective of this, the PDCAAS measurement reveals that sorghum protein is highly inadequate for all age groups, being less than one-third of the maximum value of 1.0 of proteins such as casein and egg white (WHO/FAO/UNU Expert Consultation, 2007). The value of the PDCAAS is that if the score is 1.0, policy planners are assured that the mg amino acids per g protein in that food meets 100% of the indispensable amino acid requirements for an age group. Protein requirements are based on body mass among other factors, so the absolute amount of protein required for adults will exceed that required for children, even though the amino acid requirements per kilogram body weight are greater for infants and children than for adults (Table 2.3) and that is the rationale for different amino acid patterns for PDCAAS values (Table 2.4). A thorough discussion of the relative requirements for growth versus maintenance for different ages is given in the 2002 WHO/FAO/UNU Expert Consultation (2007) report. Another application of the PDCAAS calculation is that one can easily determine how much of a food will be required in a day to meet the amino acid requirements for an individual. For that calculation, the quantity of protein in a food must be considered.

IV. SORGHUM PROTEIN QUALITY

A. Protein content and composition

The average protein content of sorghum grain is about 11% and ranges between 7% and 16% (Serna-Saldivar and Rooney, 1995). This percentage is in the same range as other major cereals, including wheat, maize, rice, barley, and pearl millet (Table 2.5). However, the protein quality of sorghum is generally substantially inferior to them, with the lysine content of its protein being substantially lower, 35–90% of the other cereals (Table 2.5). The lower lysine content occurs because the major proteins of sorghum, the kafirin prolamin storage proteins, are essentially lysine-free (reviewed by Belton *et al.*, 2006). Additionally, in comparison with maize whose zein prolamins are very similar, sorghum has a relatively smaller

TABLE 2.4 AAS and PDCAAS values for sorghum calculated using the 2002 WHO/FAO/UNU Expert Consultation recommended scoring patterns for indispensable amino acids[a]

Age group reference	Lysine scoring pattern	AAS[b]	Threonine scoring pattern	AAS	Tryptophan scoring pattern	AAS	PDCAAS (based on lysine)[c]
0.5 year	57	0.35	31	1.00	8.5	1.29	0.26
1–2 years	52	0.38	27	1.15	7.4	1.49	0.28
4–18 years	48	0.42	25	1.24	6.5	1.69	0.31
>18 years	45	0.44	23	1.35	6.0	1.83	0.33

[a] WHO/FAO/UNU Expert Consultation (2007) mg/g protein requirement.
[b] Calculated from USDA (2009) National Nutrient Database for Standard Reference NDB No. 20067, where lysine is 20, threonine 31, and tryptophan 11 mg/g protein, respectively.
[c] Based on a TD of 74% (Daniel *et al.*, 1966; Hopkins, 1981).

TABLE 2.5 Protein content, protein digestibility, lysine and leucine contents and PDCAASs of normal and improved protein quality lines of sorghum, compared to wheat, maize, barley, and pearl millet (data from several sources as indicated)

	Sorghum cv. Macia[a]	Sorghum cv. P890812 (parent of ABS032)[a]	Sorghum cv. ABS032[a]	Sorghum cv. BTX 436 (parent of 04CS11249-1xTX436)[a]	Sorghum cv. 04CS11249-1xTX436[a]	Sorghum cv. P521 opaque[b]	Sorghum (USDA 20067)[c]	Wheat (hard red winter) (USDA 20072)[c]	Maize (corn white) USDA (20314)[c]	Rice (brown long grain) USDA (20037)[c]	Barley (pearled) (USDA 20006)[c]	Pearl millet[d]
	Normal	*Normal*	*High lysine, high protein digestibility transgenic biofortified sorghum*	*Normal*	*High protein digestibility mutant*	*High-lysine Mutant*	*Normal*					
Protein (g/100 g flour)	10.6 (0.0)	11.9 (0.1)	12.8 (0.2)	12.1 (0.1)	11.9 (0.0)	10.6	12.4	14.5	10.5	9.6	7.24	14.5
Protein digestibility (wet-cooked values, unless indicated otherwise) (%)	59.8[e] (0.7)	47.4[e] (4.8)	73.7[e] (2.5)	36.4[e] (1.7)	51.9[e] (5.6)	63.2[e,f] 56.7[e,g]	74[i] 59.0[e,f] 59.8[e,f] 50.6[e,g]	86[i] (not stated) 85.5[e,f]	85[h] (not stated) 85.3[e,f] (yellow maize)	89[i] (polished, not stated 83.8[e,f] (rice type not stated) 72[j]	90[j] (not stated)	74.8[e,f]
Lysine (g/100 g flour)	0.19 (0.02)	0.25 (0.01)	0.41 (0.01)	0.18 (0.02)	0.26 (0.02)	0.31	0.25	0.39	0.30	0.37	0.27	0.48
Leucine (g/100 g flour)	1.43 (0.07)	1.63 (0.02)	1.33 (0.04)	1.77 (0.12)	1.50 (0.06)	1.29	1.64	0.98	1.29	0.80	0.50	1.77
Lysine (mg/g protein)	17.9 (2.1)	21.0 (0.5)	32.0 (0.6)	14.9 (1.2)	21.8 (1.8)	29.5	20.2	26.9	28.6	38.5	37.3	33.1

Leucine (mg/g protein)	134.9 (6.3)	137.0 (1.9)	104.0 (3.2)	131.1 (9.0)	126.0 (4.9)	122	132.3	67.6	122.9	83.3	69.1	122.1
AAS (based on lysine)	0.34	0.40	0.62	0.29	0.42	0.57	0.39	0.52	0.55	0.74	0.72	0.64
PDCAAS[k]	0.21	0.19	0.45	0.10	0.22	0.36 0.32	0.29 0.23 0.23 0.20	0.44 0.44	0.47 0.47	0.66 0.62 0.53	0.65	0.48

[a] Own data, unpublished. Means and standard deviations (in parentheses) of four independent replicate analyses.
[b] Guiragossian *et al.* (1978), except where indicated otherwise.
[c] USDA (2009) National Nutrient Database for Standard Reference, except where indicated otherwise.
[d] Serna-Saldivar and Rooney (1995), except where indicated otherwise.
[e] *In vitro* pepsin method.
[f] Mertz *et al.* (1984).
[g] Axtell et al. (1981).
[h] True digestibility (FAO/WHO Expert Consultation, 1991).
[i] Hopkins (1981).
[j] South African Department of Health (2002).
[k] PDCAAS calculated as protein digestibility × AAS for 3–10-year old children (based on lysine) using the WHO/FAO/UNU Expert Consultation (2007), using lysine and protein digestibility values in this table in order given.

germ and hence a lower level of the lysine-rich germ proteins (Taylor and Schüssler, 1986). Hence, the AAS for lysine in sorghum is only between approximately 35% and 44% of the 2002 WHO/FAO/UNU Expert Consultation (2007) recommendations for the various age groups.

Sorghum protein also appears to have low levels of the indispensable amino acids threonine and tryptophan and high levels of leucine (reviewed by Klopfenstein and Hoseney, 1995). However, as shown in Table 2.4, contrary to earlier suggestions (Klopfenstein and Hoseney, 1995), threonine does not seem to be deficient in sorghum protein. In sorghum, there is a low ratio of tryptophan relative to leucine. The leucine content of sorghum protein, approximately 13 g/100 g, is approximately twice the 2002 WHO/FAO/UNU Expert Consultation (2007) scoring pattern. Tryptophan is believed to have a sparing effect on niacin and the levels of leucine in some sorghum diets may impair this sparing effect and result in the occurrence of pellagra (reviewed by Klopfenstein and Hoseney, 1995). Evidence from rat feeding trials supports the pellagra risk theory concerning sorghum diets (Salter *et al.*, 1985). However, Young and Fukagawa (1988) disputed this risk after analysis of data from several studies.

B. Protein digestibility

A second and important issue with regard to sorghum protein quality is that the digestibility of sorghum proteins is lower than that of maize (Elmalik *et al.*, 1986; reviewed by Duodu *et al.*, 2003), despite the fact that the proteins are very similar. Additionally, and of particular significance, sorghum protein digestibility is substantially reduced after wet cooking (Axtell *et al.*, 1981; Taylor and Taylor, 2002), as occurs when sorghum flour is for example made into porridge, the most common form of sorghum foods in Africa. Duodu *et al.* (2003) showed that the reduction of *in vitro* protein digestibility that occurs when sorghum is wet cooked has been found by many workers to be substantially greater than that which occurs when maize is wet cooked. As a consequence, sorghum foods have much lower protein digestibility than maize, wheat, rice, and pearl millet foods (Mertz *et al.*, 1984).

Research over more than 20 years has provided compelling evidence that, although the causes of the reduction in sorghum protein digestibility are multifactorial (Duodu *et al.*, 2003), the major cause is cross-linking of the kafirin prolamin proteins due to disulfide bonding (Ezeogu *et al.*, 2005; Hamaker *et al.*, 1987; Rom *et al.*, 1992). Disulfide bonding seems to specifically involve the cysteine-rich γ- and β-kafirin species (Oria *et al.*, 1995) which are concentrated at the surface of the endosperm protein bodies, the organelles of kafirin storage (Shull *et al.*, 1992). The cross-linking

appears to render the major kafirin species, α-kafirin, which is located in the center of the protein bodies (i.e., beneath the γ-kafirin), less accessible to protein hydrolysis. Additionally, the kafirin proteins seem to undergo a more severe change in secondary structure on cooking than zeins, from α-helical to β-sheet conformation, which also seems to adversely affect their digestibility (Emmambux and Taylor, 2009).

The poor protein digestibility of sorghum seems to relate directly to its inferior human nutritional value, although not surprisingly in view of ethical issues, there has been very limited controlled experimental research in this area. Probably the clearest demonstration was the work of MacLean *et al.* (1981). The authors fed children, average age of 17 months who were recovering from PEM, a wet-cooked whole grain sorghum-based diet. Four varieties of sorghum were used, two normal (21–22 mg lysine/g protein) and two high-lysine (29–30 mg lysine/g protein). Because of poor weight gain (or weight loss) and inadequate nitrogen retention, most of the children did not complete the 7–9 day experimental diet sequence. The mean absorption and retention of nitrogen from 26 6-day sorghum dietary periods were 46% and 14% of intake, compared with preceding casein mean control values of 81% and 49%. Of particular relevance is the fact that there were no differences between the high-lysine and normal-lysine sorghum diets. The authors also compared these findings with other comparable data for wheat, rice, potato, and maize diets where the absorption and retention of nitrogen ranged from 66% to 81% and 20% to 34%, respectively. Further, the children's stool weight and energy losses for the sorghum diets were 2.5–3 times those of the casein diets and substantially higher than values from wheat, rice, potato, and maize diets. Additionally, the work showed that with all the sorghum diets, 3-h postprandial levels of plasma lysine did not increase compared to fasting levels and declined compared to fasting levels at 4 h, indicative of lysine deficiency. A later study (MacLean *et al.*, 1983) appeared to give contradictory results in that children fed a diet containing sorghum that had been decorticated (debranned) and processed by extrusion cooking showed very much higher nitrogen absorption and higher nitrogen retention than in the previous study. These findings have to be seen in context. Subsequent work showed that extrusion cooking (essentially a dry type of cooking), unlike wet cooking, does not reduce protein digestibility (Hamaker *et al.*, 1994) and confirms that removal of the outer layers of the sorghum grain improves protein digestibility (reviewed by Duodu *et al.*, 2003). However, at-risk people consume conventionally wet-cooked sorghum and often it is prepared from milled whole grain (personal observations).

Further, the findings of MacLean *et al.* (1981) confirm earlier work. Kurien *et al.* (1960) replaced rice in the diet of seven normal boys, aged

10–11, with various proportions of sorghum and found that the apparent protein digestibility of the diet fell from 75% to 69% to 64% to 55% as the proportion of sorghum increased from 0–25% to 50–100%. Nicol and Phillips (1978)fed adult Nigerian men diets based on sorghum, cassava, and rice and compared them to egg protein. The true digestibility of the sorghum diet was found to be lower than that of any of the others and its BV was superior only to that of cassava. The evidence of these studies was summed up by Millward (1999) in a review of the nutritional value of plant-based diets in relation to human requirements, who stated ''digestibility does seem to present a problem for many mixed plant-based diets and some cereals (e.g., millet [apparently referring to finger millet (also known as ragi) (*Eleucine coracana*)] and sorghum), and this may be a major problem for children in the developing countries.''

Results from rat feeding experiments, however, have not always agreed with the human studies. Axtell *et al*. (1981) using three of the four same sorghum varieties as MacLean *et al*. (1981) found similarly high protein digestibility (N consumed minus N in feces) with all gruels made from all the sorghums, both normal and high lysine. Further, the protein digestibility of the gruels was similar to that which had been found earlier in their laboratory when rats were fed uncooked sorghum flours. They proposed that the young rat is much more efficient at digesting sorghum proteins than children and therefore is not a good model. The findings of Axtell *et al*. (1981) are explained by a comprehensive sorghum food rat feeding study by Bach Knudsen and coworkers (Bach Knudsen and Munck, 1985; Bach Knudsen *et al*., 1988a,b; Eggum *et al*., 1983). These authors fed rats diets comprising neutral, acidic, and alkaline porridges made from non-tannin and polyphenol-rich (tannin) sorghums, and the uncooked sorghum flours. With all the types of porridges, there was a substantial decrease in TD and generally an increase in BV compared to the uncooked flours, with a great increase with high-tannin sorghum porridges. Further, with the high-tannin sorghum porridge diets, there was a substantial reduction in net protein utilization (NPU) but no effect with the non-tannin and low-tannin sorghum porridge diets. The authors attributed the reduction in TD to the kafirin proteins being rendered unavailable by cooking. They proposed that the higher BV was due to the undigested kafirins serving as a nitrogen source for bacteria in the rat hind gut. This, plus fermentation by the rat of resistant starch formed during sorghum processing, was suggested as the reasons for the difference in sorghum food feeding values between rats and people. The authors further proposed that the reason that the effects of the nutritional parameters were exacerbated in rats fed the high-tannin sorghum diets was due to complexation between the tannins and kafirins, the net effect being a change in nitrogen excretory routes from the urine to the feces.

V. RESEARCH TO IMPROVE SORGHUM PROTEIN QUALITY

Research to improve sorghum protein quality has been ongoing since the 1970s, especially at Purdue University. Singh and Axtell (1973) identified high lysine lines from Ethiopia. However, these have very poor functional quality as the grains are shrunken. By chemical mutagenesis of normal sorghum using diethyl sulfate, a high-lysine sorghum mutant called P721-opaque was developed. This has up to 60% higher lysine content due to a reduction in the relative amount of kafirins (Guiragossian *et al.*, 1978). However, as described, when both of these types of high-lysine sorghums were used in human feeding trials, there was no difference in performance between them and normal sorghums (MacLean *et al.*, 1981). As a result of the evidence that low protein digestibility is the major cause of sorghum's poor nutritional performance, improved protein digestibility types were sought. By crossing P721-opaque with normal sorghums, lines were obtained that had substantially improved protein digestibility, 10–15% higher in uncooked flour and 25% higher in cooked flour (Weaver *et al.*, 1998). These lines also have somewhat elevated levels of lysine. The improved protein digestibility appears to be due to change in the shape of the kafirin protein bodies from spherical to invaginated (Fig. 2.1) (Oria *et al.*, 2000). This change in shape results in the γ-kafirin species being concentrated at the bottom of invaginations where they should not interfere with the digestion of the α-kafirin. Tesso *et al.* (2006) identified a novel sorghum mutant with both high protein digestibility and high-lysine traits, and a relatively hard endosperm. This mutant was an F6 generation of crosses between P721-opaque and hard endosperm sorghum lines. It has some 44% higher lysine than normal

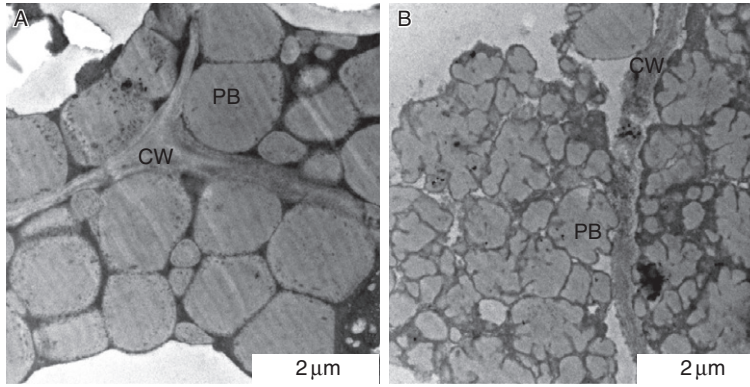

FIGURE 2.1 Transmission electron micrographs of protein bodies from (A) normal sorghum and (B) high protein digestibility mutant sorghum. PB = kafirin protein body, CW = cell wall.

sorghum and 20% higher protein digestibility. Very unusually, the grains had areas of densely packed starch granules without the usual endosperm protein matrix between them. The denser structure seemed to be responsible for the relative hardness of the grain.

Data on the *in vivo* effects of the high protein digestibility sorghum are very limited, but what there is does not show any nutritional improvement. Nyannor *et al*. (2007) investigated the effects of two different high protein digestibility sorghum lines, and normal sorghum and maize-based diets on pigs (sorghum or maize only in the diets) and broiler chickens (sorghum or maize, plus soya in the diets). It was found with the pigs that there was no difference in the apparent ileal or total tract digestibility of dry matter, energy, or nitrogen among the diets. Further, the estimated digestible energy did not differ among the diets. With the chickens, there was no difference in ileal digestibility for any of the nutrients among the different diets. Total tract nitrogen retention was better with maize than with any of the sorghums, although one of the high protein digestibility lines was better in this respect than the other and normal sorghums. There was no difference in the apparent metabolizable energy content among any of the sorghum diets but metabolizable energy was lower than in the maize diet, except for the one high protein digestibility line.

The findings of Nyannor *et al*. (2007) and those of MacLean *et al*. (1981) are consistent, showing the need to develop sorghum lines that have both substantially improved protein quality and digestibility. The ABS project is employing recombinant DNA technology to improve both sorghum lysine content and wet-cooked protein digestibility. These improvements are being achieved by suppressing the synthesis of specific kafirin species that are very low in lysine and that are responsible for poor protein digestibility (i.e., suppression of the synthesis of various combinations of α-, γ-, and δ-kafirins) using RNA interference (RNAi) technology (Jung, 2008), as has been demonstrated with the maize zein prolamins (reviewed by Shewry, 2007). Additionally, where appropriate, the synthesis of lysine-rich proteins, such as HT12, an analog of barley hordothionin, is being expressed (Zhao *et al*., 2003) and the catabolism of lysine by the enzyme lysine ketoreductase (LKR) is suppressed. Sorghum transformation is brought about using a ''super-binary'' *Agrobacterium* vector (Zhao *et al*., 2003). Preliminary data indicate that the kafirin protein bodies in the endosperm of early transgenic biofortified sorghum lines are irregular in shape, that is similar to those of the high protein digestibility mutant (Fig. 2.1). The irregular shape is presumably a reflection of suppression of kafirin synthesis.

Table 2.5 compares the protein quality in terms of lysine, leucine, protein digestibility, and PDCAAS of different types of sorghum, including an early transgenic biofortified sorghum line and other major cereals:

wheat, maize, rice, barley, and pearl millet. It should be noted that most of the protein digestibility values were obtained using the *in vitro* pepsin method developed by Chibber *et al*. (1980). As can be seen, the *in vitro* protein digestibility values from the Purdue University group (Axtell *et al*., 1981; Mertz *et al*., 1984) seem to be in good agreement with the FAO/WHO Expert Consultation (1991) TD values, which came from Hopkins (1981). The only exception is the sorghum value of 74%, which in turn came from Daniel *et al.* (1966), which is rather higher. As stated earlier, many factors affect sorghum protein digestibility, these include exogenous factors such as grain organizational structure, polyphenols, phytic acid, cell wall components, and starch (reviewed by Duodu *et al*., 2003), which can vary among individual samples. In fact, in the discussion following the conference reading of the Hopkins (1981) paper, reference was made to the effect of various factors on protein digestibility and there was some criticism of these factors not being taking into account (Hopkins, 1981). In response, Hopkins stated with specific reference to sorghum and millet ''with some of the largely vegetarian diets in some of the developing countries, it is a different story [i.e., different from the situation with regard to USA diets] and protein digestibility is of concern''. Of relevance to this, Hopkins (1981) reported data that the true digestibility of sorghum diets of Indian children was 66%, similar to values for finger millet (ragi), 65–68%.

MacLean *et al*. (1981) found nitrogen absorption in sorghum diets to be $46 \pm 17\%$, which is of the same order as the Purdue pepsin *in vitro* digestibility values (Axtell *et al*., 1981; Mertz *et al*., 1984), and on this basis, Axtell et al. (1981) stated that the pepsin *in vitro* method gave similar values to those obtained in human studies. With regard to the accuracy of our own sorghum protein digestibility data in Table 2.5, it is important to note that our *in vitro* pepsin protein digestibility results seem to be in close agreement with those of the Purdue group (Axtell *et al*., 1981; Mertz *et al*., 1984). For example, Macia, a white tan-plant sorghum, had a protein digestibility of 59.8%, the same as Dabar another white tan-plant sorghum (Mertz *et al*., 1984).

Table 2.5 shows that the lysine content of transgenic biofortified sorghum protein was 52–115% higher than that of normal sorghums, 47% higher than the high protein digestibility mutant, and 8% higher than the high-lysine mutant. In comparison with the other major cereals, the protein lysine content of transgenic biofortified sorghum was somewhat higher than that of maize and wheat, similar to pearl millet (*Pennisetum glaucum*), but lower than that of pearled barley and rice. Thus, the AAS, based on protein lysine content, of transgenic biofortified sorghum was some 50–100% higher than that of normal sorghum and high protein digestibility sorghum, and slightly higher than that of the high-lysine mutant, maize, and wheat, similar to that of pearl millet but lower than

that of pearled barley and rice. The protein leucine content of transgenic biofortified sorghum was some 20% lower than in normal sorghum, and 15–17% lower than in the high protein digestibility and high-lysine mutants.

The wet-cooked *in vitro* protein digestibility of transgenic biofortified sorghum was 23–102% higher than that of the normal sorghums and 8% and 42% higher, respectively, than the corresponding levels in the high-lysine sorghum and high protein digestibility sorghum mutants. Importantly, the wet-cooked protein digestibility of transgenic biofortified sorghum was raised to levels approaching those of the other major cereals. In this regard, it is important to point out that this early version of transgenic biofortified sorghum was in a low-tannin sorghum (Type 2 sorghum) background. The tannins in tannin sorghum reduce protein digestibility, both *in vitro* (Chibber *et al*., 1980) and *in vivo* (Bach Knudsen *et al*., 1988a), primarily through complexing with the proline-rich kafirins (Butler *et al*., 1984; Taylor *et al*., 2007). This kafirin–tannin complexation is indicated by the relatively low protein digestibility of the transgenic biofortified sorghum parent cultivar P890812. Thus, it is probable that the wet-cooked protein digestibility will be improved somewhat further when the protein quality traits are in non-tannin sorghum lines. The effect of improving both protein lysine content and protein digestibility on PDCAAS is substantial. The PDCAAS of transgenic biofortified sorghum for 1–2-year-old children is double that of normal sorghum and the high protein digestibility sorghum, and 25–40% higher than the high-lysine sorghum. Significantly, the PDCAAS of transgenic biofortified sorghum is the same level as maize, wheat, and pearl millet.

VI. WILL PROTEIN BIOFORTIFICATION OF SORGHUM MAKE A DIFFERENCE?

The limited research that has been carried out on the effects of protein and lysine biofortication/fortication of cereal staples on the nutritional status of children has yielded mixed results. Daniel *et al.* (1966) carried out a short-term study where a sorghum-rich diet was fortified with lysine or lysine plus threonine in the form of free amino acids (i.e., not in proteins) and given to 11–12-year-old girls from a low income group in India. Fortification with lysine resulted in a small increase in nitrogen retention, BV, and NPU. Fortification with lysine plus threonine resulted in a much larger improvement in these parameters. It is noteworthy that the nitrogen retention of the unfortified sorghum-rich diet was only 8.6% compared to the referenced skim milk-rich diet of 32.9%. Supplementation with lysine plus threonine increased nitrogen retention to 21.8%.

King *et al*. (1963) fortified wheat bread with the indispensable amino acid lysine and undertook a yearlong study with chronically undernourished children in Haiti aged 6–18 years. The experimental design was such that there were eight groups of children divided according to age and sex. A significant improvement in corpuscular hemoglobin concentration was observed for three of the eight groups receiving the lysine-fortified bread compared to the unfortified bread group and a significant increase in height was observed in two of the groups. However, compared to a control group, there was only an increase in hemoglobin concentration in two of the groups. Significantly, the authors suggested that other limiting factors such as threonine and vitamin A and riboflavin would need to be addressed if optimal growth and hemoglobin responses were to be obtained. Subsequently, in the 1970s, three major, long-duration studies were undertaken where cereal-rich diets (rice, wheat, and maize) of people in developing countries were fortified with lysine and other vitamins and minerals (reviewed by Pellett and Ghosh, 2004). None of the studies reported any significant health benefits, and a task force investigating the reasons found that there were serious flaws in the design, methods, or analysis in the studies (Latham, 1988).

More recently, two large, long-term, double-blind studies were undertaken by Scrimshaw and coworkers, in which families in Pakistan and China were given wheat flour fortified with lysine (0.6 g/100 g and 0.3 g/100 g, respectively) or control flour to make into bread-type products as their major dietary staple (Hussain *et al.*, 2004; Zhao *et al*., 2004). A range of anthropometric measurements and blood tests were performed and the results analyzed according to men, women, and children. In both studies, the height and weight increases of the children in the lysine-fortified flour group were significantly higher than in the control groups. In the Pakistan study (Hussain *et al.*, 2004), transferrin levels increased significantly in men, women, and children receiving lysine-fortified flour and hemoglobin increased in the women. In the China study (Zhao *et al*., 2004), hemoglobin levels were not affected. However, perhaps of importance with respect to HIV/AIDS, CD3 T cell levels increased significantly in women and children receiving the lysine-fortified flour and the increases in the three immunoglobulins IgG, IgA, and IgM were highly significant ($p < 0.01$) in the children.

With respect to the mixed findings on fortification of cereals with free lysine, attention was drawn by the 2002 WHO/FAO/UNU Expert Consultation (2007) to the issue of Maillard reactions between amino acids, especially lysine, and sugars that can occur during food processing. Maillard reactions adversely affect the bioavailability of amino acids. Lysine, as a free amino acid, is particularly susceptible to loss through the Maillard reaction (Ajandouz and Puigserver, 1999).

The work of MacLean *et al*. (1981) where there was no difference in the effects of high-lysine sorghum compared to normal sorghum has already been described. The findings of this study contrast sharply with those of Graham *et al*. (1990) on high-lysine maize. These authors worked with young children in Peru, aged 13–29 months, who were recovering from malnutrition. The children were fed a diet comprising solely a porridge of Quality Protein Maize (QPM) for a period of up to 90 days. QPM is a high-lysine mutant and also contains elevated levels of tryptophan and lower levels of leucine. The lysine level in the QPM cultivars used was 38–40 mg/g protein, that is, 20% higher than in current transgenic biofortified sorghum lines (Table 2.5). In the diets, QPM provided all the protein and lipid and 90% of the energy, the remainder coming from sucrose. The diet was also supplemented with a full complement of vitamins. The responses of the children on the QPM diet were compared to those of similar children receiving a modified cow's milk formula (CMF). Significantly, the weight gain and height growth were the same between the QPM and CMF groups. The QPM showed a small reduction in serum albumin levels and the plasma-free total essential amino acids and ratio of these to total essential amino acids was lower in the QPM group. With reference to this, the authors stated that the lower plasma albumin levels were a characteristic of vegetable (plant) protein diets. Concerning the dramatic difference in response of the children fed high-lysine maize in this study to those receiving high-lysine sorghum in the study of MacLean *et al*. (1981), this seems to be related to the fact that, when sorghum is wet cooked, its protein digestibility is considerably reduced, whereas this is not the case with maize, as described above. In this respect, it is relevant that, in another study by Graham and coworkers involving feeding recovering malnourished children QPM, it was found that the children fed QPM had significantly higher nitrogen retention than children fed normal maize ($p < 0.01$) (Graham *et al*., 1989). Hence, the fact that transgenic biofortified sorghum has improved protein digestibility as well as increased lysine is probably critical to it making a difference to children's nutritional status.

A. Potential impact of biofortified sorghum

The potential impact of biofortified sorghum can be evaluated by taking Burkina Faso as an example. Sorghum is the most important staple in Burkina Faso, accounting for 40% of cereal production (FAOSTAT, 2007). There are serious child nutrition and health problems in Burkina Faso. According to statistics from the Ministry of Health, in 2007, the average incidence of malnutrition for children under the age of 5 years was 10.9%, with 1.4% of children being severely malnourished (Burkina Faso Ministry of Health, 2008). With respect to malnutrition-related diseases

in the children, 5200 cases of anemia and 591 cases of xerophthalmia were reported and PEM accounted for 4% of the principal causes of hospital admissions.

With respect to protein intake in Burkina Faso, in 2003–2005, sorghum contributed 28%, pearl millet 19%, maize 13%, groundnuts 11%, pulses 9%, rice 4%, meat 6%, milk 2%, and wheat 1% (FAO, 2008b). The total cereal contribution to protein intake is 65% with PDCAAS values ranging from approximately 0.28 (sorghum) to 0.66 (rice) (Table 2.5), using the 2002 WHO/FAO/UNU Expert Consultation (2007) reference pattern for 1–2-year-old children. Only 8% (milk + meat) of the dietary protein intake sources has a PDCAAS of 1.0 (FAO/WHO Expert Consultation, 1991), yet the protein recommendations are based on a PDCAAS of 1.0. Thus, with respect to sorghum meeting 28% of a 2-year-old child's (median weight 12.3 kg; FAO/WHO/UNU Expert Consultation, 1985) safe requirement of 0.97 g protein/kg body weight per day (WHO/FAO/UNU Expert Consultation, 2007), that is 3.3 g protein, the child would have to consume

$$3.3 \times \frac{100}{12.4^{a}} \times \frac{1.00}{0.28^{b}} = 90\,\text{g sorghum grain}$$

[a] USDA (2009) National Nutrient Database for Standard Reference protein content of sorghum grain

[b] PDCAAS of normal sorghum (Table 2.5)

The solids content of the sorghum Tô, the gel-like porridge, consumed in Burkina Faso and other Sahel countries, is approximately 20% (Scheuring *et al.*, 1982). Thus, the child would have to consume some 475 g of Tô made from normal sorghum per day, a very large amount. The situation would be even worse in the case where sorghum was the sole cereal consumed, which is often the case in the period before harvest where other foodstuffs have all been consumed, as sorghum's PDCAAS is only approximately half that of the other cereals (Table 2.5). Then the child would have to consume approximately 1.1 kg of sorghum Tô per day, equivalent to 9% of its body weight. The requirement in high cereal diets for extremely high cereal intakes to satisfy children's protein requirement is in agreement with analysis by Millward (1999) of dietary data from West Bengal, India, published by Pellett (1996). In the West Bengal diet, cereals supplied 90% of protein. Millward showed that the AAS for all preschool children was less than 1.0. He concluded that the children, when weaned, would need either extra milk or pulses if the energy density of the diet did not allow them to increase food intake sufficiently. In this context, it should be noted that the PDCAAS of milk and pulses are

much higher, 1.00 and 0.77 (chickpea) for 2-year-old children (WHO/FAO/UNU Expert Consultation, 2007) than those of cereals (Table 2.5).

The key issue is thus that with transgenic biofortified sorghum, the fact that PDCAAS is doubled means that half the amount of Tô or other sorghum cereal food needs to be consumed by the children in order to meet their safe protein intake. This is based on the assumption that the diet sufficiently meets their energy requirements. On the basis of the findings of Graham *et al*. (1990) where QPM porridge met all the protein requirements and 90% of the energy requirements of children of this age, it would seem to be the case.

As indicated above, dietary protein cannot be considered in isolation from dietary micronutrients. Widespread micronutrient malnutrition is acknowledged in Africa. The major global micronutrient deficiencies identified in a United States Institute of Medicine publication (Committee on Micronutrient Deficiencies, 1998) were vitamin A, iron, iodine, and zinc. It was pointed out that the high intake of phytate in grain-based diets affects mineral status. Among the various options for addressing micronutrient deficiencies, supplementation, fortification, and food-based approaches, the food-based approach attempts to correct the underlying causes of micronutrient deficiencies and is most effective where there is widespread availability, variability, adequacy, and acceptability. Periodic micronutrient supplementation programs have been successful but are not sustainable (Committee on Micronutrient Deficiencies, 1998). Tang *et al*. (2009) demonstrated that the biofortified Golden Rice, which contains 1.6–35 μg β-carotene (provitamin A) per gram dry rice, is effectively converted to vitamin A in humans. With respect to transgenic biofortified sorghum, preliminary data from early generation lines show reduced levels of phytate, indicating higher iron and zinc bioavailability and similar levels of provitamin A to Golden Rice (Dr Zuo-Yu Zhao, research scientists, Pioneer Hi-Bred International). As an indication of the potential of provitamin A biofortification, Van Jaarsveld *et al.* (2005) found in a controlled experiment that children consuming orange-fleshed sweet potato containing approximately 100 μg β-carotene per gram showed improved vitamin A status compared to those consuming normal white-fleshed sweet potato. Through consumption of the orange-fleshed sweet potato, there was an increase from 78% to 87% in the percentage of children with normal vitamin A status.

VII. CONCLUSIONS

Biofortified sorghum, developed using recombinant DNA technology, has substantially improved protein quality in terms of lysine content and wet-cooked protein digestibility compared to normal sorghum.

In terms of PDCAAS, it is similar to other major cereals. In addition, the biofortified sorghum will have higher bioavailability of iron and zinc and significant levels of provitamin A. Thus, cultivation and consumption of biofortified sorghum, Biosorghum, could provide a sustainable and broadly comprehensive solution to the protein and certain micronutrient deficiencies suffered by young children in sub-Saharan African countries that are dependent on cereals as their staple food.

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REFERENCES

Ajandouz, E. H. and Puigserver, A. (1999). Nonenzymatic browning reaction of essential amino acids: Effect of pH on caramelization and Maillard reaction kinetics. *J Agric. Food Chem.* **47**, 1786–1793.

AOAC International (2000). AOAC Official method 991.29 True protein digestibility of foods and food ingredients: Rat bioassay first action 1991. *In* ''Official Methods of AOAC International'', (W. Horwitz, Ed.), 17th edn, Vol. II, pp. 66–67. AOAC International, Gaithersburg, MD (Chapter 45).

Axtell, J. D., Kirleis, A. W., Hassen, M. M., D'Croz Mason, N., Mertz, E. T., and Munck, L. (1981). Digestibility of sorghum proteins. *Proc. Natl. Acad. Sci. USA* **78**, 1333–1335.

Bach Knudsen, K. E. and Munck, L. (1985). Dietary fibre contents and compositions of sorghum and sorghum-based foods. *J. Cereal Sci.* **3**, 153–164.

Bach Knudsen, K. E., Munck, L., and Eggum, B. O. (1988a). Effect of cooking, pH and polyphenol level on carbohydrate composition and nutritional quality of a sorghum (*Sorghum bicolor* (L.) Moench) food, ugali. *Br. J. Nutr.* **59**, 31–47.

Bach Knudsen, K. E., Kirleis, A. W., Eggum, B. O., and Munck, L. (1988b). Carbohydrate composition and nutritional quality for rats of sorghum tô prepared from decorticated white and whole grain red flour. *J. Nutr.* **118**, 588–597.

Belton, P. S., Delgadillo, I., Halford, N. G., and Shewry, P. R. (2006). Kafirin structure and functionality. *J. Cereal Sci.* **44**, 272–286.

Biocassava Plus (2010). www.biocassavaplus.org (Accessed January 2010).

Biosorghum (2010). www.biosorghum.org (Accessed January 2010).

Burkina Faso Ministry of Health (2008). Annuiare Statistique Santé – 2007. Ministere de la Santé, Ouagadougou.

Butler, L. G., Riedl, D. J., Lebryk, D. G., and Blytt, H. J. (1984). Interaction of proteins with sorghum tannin: Mechanism, specificity and significance. *J. Am. Oil Chem. Soc.* **61**, 916–920.

Chibber, B. A. K., Mertz, E. T., and Axtell, J. D. (1980). In vitro digestibility of high-tannin sorghum at different stages of dehulling. *J. Agric. Food Chem.* **26**, 160–161.

Committee on Micronutrient Deficiencies (1998). *In* ''Prevention of Micronutrient Deficiencies: Tools for Policymakers and Public Health Workers'', (C. P. Howson, E. T. Kennedy, and A. Horwitz, Eds), National Academy Press, Washington, DC.

Daniel, V. A., Leela, R., Doraiswamy, T. R., Rajalakshmi, D., Venkat Rao, S., Swaminathan, M., and Parpia, H. A. B. (1966). The effect of supplementing a poor kaffir corn (*Sorghum vulgare*) diet with L-lysine and DL-threonine on the digestibility coefficient, biological value and net utilization of proteins and retention of nitrogen in children. *J. Nutr. Diet.* **3**, 10–14.

De Blaauw, I., Deutz, N. E. P., and Von Meyenfeldt, M. F. (1996). In vivo amino acid metabolism of gut and liver during short and prolonged starvation. *Am. J. Physiol.* **270**, G298–G306.

De Onis, M., Blossner, B., Borghi, E., Morris, R., and Frongillo, E. A. (2004). Methodology for estimating regional and global trends of child malnutrition. *Int. J. Epidemiol.* **33**, 1260–1270.

Doggett, H. (1988). Sorghum, 2nd edn. Longman Scientific and Technical, London, pp. 1–3.

Duodu, K. G., Taylor, J. R. N., Belton, P. S., and Hamaker, B. R. (2003). Mini review: Factors affecting sorghum protein digestibility. *J. Cereal Sci.* **38**, 117–131.

Eggum, B. O., Monowar, L., Bach Knudsen, K. E., Munck, L., and Axtell, J. (1983). Nutritional quality of sorghum and sorghum foods from Sudan. *J. Cereal Sci.* **1**, 127–137.

Elmalik, M., Klopfenstein, C. F., Hoseney, R. C., and Bates, L. S. (1986). Digestibility and nutritional value of sorghum grain with contrasting kernel characteristics. *Nutr. Rep. Int.* **34**, 811–821.

Emmambux, M. N. and Taylor, J. R. N. (2009). Properties of heat-treated sorghum and maize meals and their prolamin proteins. *J. Agric. Food Chem.* **57**, 1045–1050.

Ezeogu, L. I., Duodu, K. G., and Taylor, J. R. N. (2005). Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *J. Cereal Sci.* **42**, 33–44.

FAO (2008a). Food Security Statistics. Food Consumption, Dietary Energy, Proteins, Fat. www.fao.org/economic/ess/foodsecurity-statistics/en/ (Accessed January 2010).

FAO (2008b). Food Security Statistics. Consumption Pattern of Main Food Crops-Dietary Protein. www.fao.org/economic/ess/foodsecurity-statistics/en/ (Accessed January 2010).

FAO (2009). Food Security Statistics. Child Nutrition Status. www.fao.org/economic/ess/foodsecurity-statistics/en (Assessed January 2010).

FAO/WHO (1973). Energy and protein requirements. Food and Agriculture Organization and World Health Organization, Rome and Geneva, Report of a joint FAO/WHO Ad Hoc Expert Committee. FAO Nutrition meetings Report Series, No. 52, and WHO Technical Report Series, No 522.

FAO/WHO Expert Consultation (1991). Protein Quality Evaluation. Food and Agriculture Organization, Rome, Report of Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Paper 51.

FAO/WHO/UNU Expert Consultation (1985). Energy and Protein Requirements. World Health Organization, Geneva Report of a Joint FAO/WHO/UNU Expert Consultation. World Health Organization Technical Report Series 724.

FAOSTAT (2007). FAOSTAT-Agriculture, Production, Crops. http://faostat.fao.org/ (Accessed November 2009).

Frost, H. M. (1997). On our age-related bone loss: Insights from a new paradigm. *J. Bone Miner. Res.* **12**, 1–9.

Golden Rice (2010). www.goldenrice.org (Accessed January 2010).

Graham, G. G., Lembcke, J., Lancho, E., and Morales, E. (1989). Quality protein maize: Digestibility and utilization by recovering malnourished infants. *Pediatrics* **83**, 416–421.

Graham, G. G., Lembcke, J., and Morales, E. (1990). Quality-protein maize as the sole source of dietary protein and fat for rapidly growing young children. *Pediatrics* **85**, 85–91.

Grand Challenges in Global Health (2010). Optimisation of Bioavailable Nutrients in Transgenic Bananas. www.grandchallenges.org/ImproveNutrition/Challenges/NutrientRichPlants (Accessed January 2010).

Guiragossian, V., Chibber, B. A. K., Van Scoyoc, S., Jambunathan, R., Mertz, E. T., and Axtell, J. D. (1978). Characteristics of proteins from normal, high lysine, and high tannin sorghums. *J. Agric. Food Chem.* **26**, 219–223.

Guyton, A. C. and Hall, J. E. (1996). Textbook of Medical Physiology, 9th edn W.B. Saunders Company, Philadelphia, PA, pp. 863, 980, 880, 881.

Hamaker, B. R., Kirleis, A. W., Butler, L. G., Axtell, J. D., and Mertz, E. T. (1987). Improving the *in vitro* protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci. USA* **84**, 626–628.

Hamaker, B. R., Mertz, E. T., and Axtell, J. D. (1994). Effect of extrusion on sorghum kafirin solubility. *Cereal Chem.* **71**, 515–517.

Hansen, R. D., Raja, C., and Allen, B. J. (2000). Total body protein in chronic diseases and in aging. *Ann. N Y Acad. Sci.* **904**, 345–352.

Henley, E. C. and Kuster, J. M. (1994). Protein quality evaluation by protein digestibility-corrected amino acid scoring. *Food Technol.* **48**(4), 74–77.

Hoffer, L. J. (1994). Starvation. *In* ''Modern Nutrition in Health and Disease'', (M. E. Shils, J. A. Olson, and M. Shike, Eds), 8th edn, pp. 927–949. Lea & Febiger, Philadelphia, PA.

Hopkins, D. T. (1981). Effects of variation in protein digestibility. *In* ''Protein Quality in Humans: Assessment and in vitro Estimation'', (C. E. Bodwell, J. S. Adkins, and D. T. Hopkins, Eds), pp. 169–193. AVI, Westport, CT.

Hussain, T., Abbas, S., Khan, M. A., and Scrimshaw, N. S. (2004). Lysine fortification of wheat flour improves selected indices of the nutritional status of predominantly cereal-eating families in Pakistan. *Food Nutr. Bull.* **25**, 114–122.

ICRISAT (2009). Sorghum. www.icrisat.org/newsite/crop-sorghum.htm (Accessed November 2009).

Institute of Medicine of the National Academies (2005). Dietary Reference Intakes for Energy, Carbohydrate, Fiber Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. National Academies Press, Washington, DC, www.nap.edu (Accessed May, 2009).

Jung, R. (2008). Grain quality improvement through altered expression of seed proteins. United States Patent Application 20080134361.

Keys, A., Brozek, J., Henshel, A., Mickelsen, O., and Longstreet, T. H. (1950). The Biology of Human Starvation. University of Minnesota Press, Minneapolis, MN.

King, K. W., Sebrell, W. H., Jr., Severinghaus, E. L., and Storvick, W. O. (1963). Lysine fortification of wheat bread fed to Haitian School children. *Am. J. Clin. Nutr.* **12**, 36–47.

Klopfenstein, C. F. and Hoseney, R. C. (1995). Nutritional properties of sorghum and the millets. *In* ''Sorghum and Millets: Chemistry and Technology'', (D. A. V. Dendy, Ed.), pp. 125–168. American Association of Cereal Chemists, St. Paul, MN.

Kotler, D. P., Tierney, A. R., and Wang, J. (1998). The magnitude of body cell mass depletion determines the timing of death from wasting in AIDS. *Am. J. Clin. Nutr.* **50**, 444–447.

Kurien, P. P., Narayanarao, M., Swaminathan, M., and Subrahmanan, V. (1960). The metabolism of nitrogen, calcium and phosphorus in undernourished children. 6. The effect of complete replacement of rice in poor vegetarian diets by kaffir corn (*Sorghum vulgare*). *Br. J. Nutr.* **14**, 339–345.

Laidlaw, S. A. and Kopple, J. D. (1987). Newer concepts of the indispensable amino acids. *Am. J. Clin. Nutr.* **46**, 593–605.

Latham, M. C. (1988). Amino Acid Fortification of Cereals: Results and Interpretation of Trials in Three Countries. *In* ''Cornell International Nutrition Monograph 20.'' Cornell University, Ithaca, NY.

Lentner, C. (1981). Units of Measurement, Body Fluids, Composition of the Body, Nutrition. *In* ''Geigy Scientific Tables,'' 8th edn. Ciba-Geigy Corporation, West Caldwell, NJ.

MacLean, W. C., Jr., López, de Romaña, G., Placko, R. P., and Graham, G. C. (1981). Protein quality and digestibility of sorghum in preschool children: balance studies and plasma free amino acids. *J. Nutr.* **111**, 128–136.

MacLean, W. C., Jr., López de Romaña, G., Castañaduy, A., and Graham, G. G. (1983). The effect of decortication and extrusion on the digestibility of sorghum by preschool children. *J. Nutr.* **113**, 2171–2177.

Mertz, E. T., Hassen, M. M., Cairns-Whittern, C., Kirleis, A. W., Tu, L., and Axtell, J. D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proc. Natl. Acad. Sci. USA* **81**, 1–2.

Millward, D. J. (1999). Meat or wheat for the next millennium? The nutritional value of plant-based diets in relation to human amino acid and protein requirements. *Proc. Nutr. Soc.* **58**, 249–260.

Nicol, B. M. and Phillips, P. G. (1978). The utilization of proteins and amino acids in diets based on cassava (*Manihot utilissima*), rice sorghum (*Sorghum sativa*) by young Nigerian men of low income. *Br. J. Nutr.* **39**, 271–286.

Nyannor, E. K. D., Adedokun, S. A., Hamaker, B. R., Ejeta, G., and Adeola, O. (2007). Nutritional evaluation of high-digestible sorghum for pigs and broiler chicks. *J. Anim. Sci.* **85**, 196–203.

Oria, M. P., Hamaker, B. R., and Shull, J. M. (1995). Resistance of sorghum α-, β- and γ-kafirins to pepsin digestion. *J. Agric. Food Chem.* **43**, 2148–2153.

Oria, M. P., Hamaker, B. R., Axtell, J. D., and Huang, C.-P. (2000). A highly digestible sorghum mutant cultivar exhibits unique fold structure of endosperm protein bodies. *Proc. Natl. Acad. Sci. USA* **97**, 5065–5070.

Pellett, P. L. (1996). World essential amino acid supply with attention to South-East Asia. *Food Nutr. Bull.* **17**, 204–234.

Pellett, P. L. and Ghosh, S. (2004). Lysine fortification: Past, present and future. *Food Nutr. Bull.* **25**, 107–113.

Picou, D., Halliday, D., and Garrow, J. S. (1966). Total body protein, collagen and non-collagen protein in infantile protein malnutrition. *Clin. Sci.* **30**, 345–351.

Polleti, S., Gruissem, W., and Sauter, C. (2004). Nutritional fortification of cereals. *Curr. Opin. Biotechnol.* **15**, 162–165.

Rand, W. M., Pellett, P. L., and Young, V. R. (2003). Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am. J. Clin. Nutr.* **77**, 109–127.

Reeds, P. J., Field, C. R., and Jahoor, F. (1994). Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *J. Nutr.* **124**, 906–910.

Rom, D. L., Shull, J. M., Chandrashekar, A., and Kirleis, A. W. (1992). Effects of cooking and treatment with sodium bisulfite on in vitro digestibility and microstructure of sorghum flour. *Cereal Chem.* **69**, 178–181.

Salter, M., Bender, D. A., and Pogson, C. I. (1985). Leucine and tryptophan metabolism in rats. *Biochem. J.* **225**, 277–281.

Scheuring, J. F., Sidibe, S., and Kante, A. (1982). Sorghum alkali tô: Quality considerations. *In* ''International Symposium on Sorghum Grain Quality'', (L. W. Rooney and D. S. Murty, Eds), pp. 24–31. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Serna-Saldivar, S. and Rooney, L. W. (1995). Structure and chemistry of sorghum and millets. *In* ''Sorghum and Millets: Chemistry and Technology'', (D. A. V. Dendy, Ed.), pp. 69–124. American Association of Cereal Chemists, St. Paul, MN.

Shewry, P. R. (2007). Improving the protein content and composition of cereal grain. *J. Cereal Sci.* **46**, 239–250.

Shull, J. M., Watterson, J. J., and Kirleis, A. W. (1992). Purification and immunocytochemical localization of kafirins in *Sorghum bicolor* (L. Moench) endosperm. *Protoplasma* **171**, 64–74.

Singh, R. and Axtell, J. D. (1973). High lysine mutant gene (hl) that improves the quality and nutritional value of grain sorghum. *Crop Sci.* **13**, 535–539.
South African Department of Health (2002). Regulations Relating to Labelling and Advertising of Foodstuffs, Regulation 1055. www.doh.gov.za/docs/regulations/2002/reg1055.html (Accessed January 2010).
Swick, R. W. and Benevenga, N. J. (1977). Labile protein reserves and protein turnover. *J. Dairy Sci.* **60**, 505–515.
Szulc, P., Beck, T. J., Marchand, F., and Delmas, P. D. (2005). Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men – the MINOS study. *J. Bone Miner. Res.* **20**, 721–729.
Tang, G., Qin, J., Dolnikowski, G. G., Russell, R. M., and Grusak, M. A. (2009). Golden rice is an effective source of vitamin A. *Am. J. Clin. Nutr.* **89**, 1776 1183.
Taylor, J. R. N. and Schüssler, L. (1986). The protein compositions of the different anatomical parts of sorghum grain. *J. Cereal Sci.* **4**, 361–369.
Taylor, J. and Taylor, J. R. N. (2002). Alleviation of the adverse effects of cooking on protein digestibility in sorghum through fermentation in traditional African porridges. *Int. J. Food Sci. Technol.* **37**, 129–138.
Taylor, J., Bean, S. R., Ioerger, B. P., and Taylor, J. R. N. (2007). Preferential binding of sorghum tannins with gamma-kafirin and the influence of tannin binding on kafirin digestibility and biodegradation. *J. Cereal Sci.* **46**, 22–31.
Tesso, T., Ejeta, G., Chandrashekar, A., Huang, C.-P., Tandjung, A., Lewamy, M., Axtell, J. D., and Hamaker, B. R. (2006). A novel modified endosperm texture in a mutant high-protein digestibility/high-lysine sorghum (*Sorghum bicolor* (L.) Moench). *Cereal Chem* **83**, 194–201.
Torun, B. and Chew, F. (1994). Protein-energy malnutrition. *In* ''Modern Nutrition in Health and Disease'', (M. E. Shils, J. A. Olson, and M. Shike, Eds), 8th edn, pp. 950–976. Lea & Febiger, Philadelphia, PA.
UNICEF (2009). The State of the World's Children. www.unicef.org (Accessed May 2009).
USDA (2009). National Nutrient Database for Standard Reference. www.nal.usda.gov/fnic/foodcomp/search (Accessed November 2009).
Van Jaarsveld, P. J., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J., and Spinnler Benadé, A. J. (2005). β-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose response test. *Am. J. Clin. Nutr.* **81**, 1080–1087.
Varmus, H., Klausner, R., Zerhouni, E., Acharya, T., Daar, A. S., and Singer, P. A. (2003). Public health: Grand challenges in global health. *Science* **302**, 398–399.
Weaver, C. A., Hamaker, B. R., and Axtell, J. D. (1998). Discovery of grain sorghum germ plasm with high uncooked and cooked in vitro protein digestibility. *Cereal Chem.* **75**, 665–670.
White, P. J. and Broadley, M. R. (2005). Biofortifying crops with essential mineral elements. *Trends Plant Sci.* **10**, 587–595.
WHO/FAO/UNU Expert Consultation (2007). Protein and Amino Acid Requirements in Human Nutrition. World Health Organization, Geneva Report of a Joint WHO/FAO/UNU Expert Consultation. World Health Organization Technical Report No. 935.
Winick, M. (1979). Hunger disease. Studies by the Jewish Physicians in the Warsaw Ghetto. John Wiley & Sons, New York.
Wolfe, R. R. (2006). The underappreciated role of muscle in health and disease. *Am. J. Clin. Nutr.* **84**, 475–482.
Wolfe, R. R. and Chinkes, D. L. (2005). Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis. 2nd edn John Wiley & Sons, New York.
Young, V. R. and Fukagawa, N. K. (1988). Amino acid interactions: A selective review. *In* ''Nutrient Interactions'', (C. E. Bodwell and J. W. Erdman, Eds), pp. 27–71. Marcel Dekker, New York.

Young, V. R., Hussein, M. A., and Scrimshaw, J. S. (1968). Estimate of loss of labile body nitrogen during acute protein deprivation in young adults. *Nature* **218**, 568–569.

Young, V. R., Scrimshaw, N. S., and Pellet, P. L. (1998). Significance of dietary protein source in world nutrition: animal and/or plant protein? *In* ''Feeding a World Population of More Than 8 Billion People: A Challenge to Science'', (J. C. Waterlow, D. G. Armstrong, L. Fowden, and R. Riley, Eds), pp. 205–212. Oxford University Press, New York.

Zhao, Z. (2007). The Africa Biofortified Sorghum Project – Applying Biotechnology to Develop Nutritionally Improved Sorghum in Africa. *In* ''Biotechnology and Sustainable Agriculture 2006 and Beyond'', (Z. Xu, J. Li, Y. Xue, and W. Yang, Eds), pp. 273–277. Springer, Dordrecht.

Zhao, Z., Glassman, K., Sewalt, V., Wang, N., Miller, M., Chang, S., Thompson, T., Catron, S., Wu, E., Bidney, D., Kebede, Y., and Jung, R. (2003). Nutritionally improved transgenic sorghum. *In* ''Plant Technology 2002 and Beyond'', (I. K. Vasil, Ed.), pp. 413–416. Kluwer, Dordrecht.

Zhao, W., Zhai, F., Zhang, D., An, Y., Liu, Y., He, Y., and Ge, K. (2004). Lysine-fortified wheat flour improves the nutritional and immunological status of wheat-eating families in northern China. *Food Nutr. Bull.* **25**, 123–129.

Zimmermann, M. B. and Hurrell, R. F. (2002). Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr. Opin. Biotechnol.* **13**, 142–145.