FINAL REPORT

Evaluating the Quality of Protein from Hemp Seed and Hemp Seed Products Through the use of the Protein Digestibility-Corrected Amino Acid Score Method

Submitted to:

Mr. Arthur Hanks,
Canadian Hemp Trade Alliance

Submitted by:

Dr. James D. House,
Department of Animal Science,
University of Manitoba,
Winnipeg, MB, R3T 2N2

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INTRODUCTION

The commercial production of hemp (Cannabis sativa L.) in Canada was permitted in 1998 following a long period of discontinuation. Traditionally, hemp was cultivated as a multi-use crop, serving as a source of fibre, food, and other products, including health care and medicinal products. Despite the utility of this crop, its cultivation in Canada was deemed illegal, due to the presence of the psychoactive compound tetrahydrocannabinal (THC) in the plant components, especially in the subspecies Cannabis sativa indica. The development of industrial hemp varieties with low levels of THC have led to reintroduction of this plant into the Canadian production systems as of 1998, provided producers obtain a license to grow the crop from Health Canada. In 2006, in excess of 20,000 hectares were licensed for industrial hemp production in Canada, with 57% of the production based in Manitoba and 30% based in Saskatchewan (Agriculture and Agrifood Canada, 2007).

Current hemp cultivars have been selected for either seed oil production, fibre production, or as dual-purpose crops. With respect to seed oil production, the concentration of hemp production in the Eastern Prairies has been concurrent with the development of hemp seed processing capacity in these regions. Seed crushing plants in Manitoba have been producing hemp oil for the industrial and human food markets. The oil content of hemp seeds is approximately 33-35% (Callaway, 2004), and the bulk of the oil extracted is through cold-pressing/extrusion-based processing. The remaining seed cake or meal has significant oil content (approx. 10%) and high (> 30%) protein content (Mustafa et al., 1999; Callaway, 2004; Silversides et al., 2005), and has been marketed into the human food arena as a source of vegetable protein. In addition to the hemp seed meal, whole hemp seed and dehulled hemp seed (hemp nuts) are found in the human food marketplace.

While data exists as to the protein content of hemp seed and hemp seed meal, there exists little information of the quality of the protein. From a nutritional standpoint, numerous factors are known to influence the quality of dietary proteins, most notably the amino acid composition and the digestibility of the protein (FAO/WHO, 1990). The amino acid composition of a plant protein can be influenced by such factors as variety/genetics, agronomic conditions such as soil fertility, and post-harvest processing effects that alter the ratio of seed components (ie: dehulling). With respect to the digestibility of the protein, the presence of antinutritional factors and high temperature processing can lead to reductions in the digestibility of proteins (Sarwar, 1997). As the hemp industry moves towards increasing the utilization of hemp seed co-products with particular reference to the protein composition, factors that influence the quality of hemp proteins must be determine in order to satisfy regulatory requirements with respect to protein content claims.

OBJECTIVES

The current study was undertaken with the following objectives:

- To determine the nutrient content of hemp seed products derived from different hemp cultivars grown in Manitoba and Saskatchewan in the 2003, 2004 and 2005 cropping years
- To determine the nutritional quality of the protein found in select hemp products, through the use of the protein digestibility-corrected amino acid score method

MATERIALS & METHODS

Sample Procurement

Thirty samples of hemp products (minimum 500 grams) were obtained from two commercial hemp receiving and crushing plants (Hemp Oil Canada, Ste. Agathe, Manitoba; Manitoba Harvest, Winnipeg, MB). The details of the hemp products are presented in Table 1, and they included 11 samples of whole hemp seed, 10 samples of hemp seed meal (cold press extraction), 6 samples of dehulled hemp seed (hemp nuts), and 3 samples of hemp seed hulls. Hemp products were derived from one of four hemp cultivars: USO14, USO 31, Crag, or Finola. USO 14 and 31 varieties are early maturing cultivars, with significant stalk yield and yield potentials of approximately 400 kg per acre (Manitoba Agriculture, Food and Rural Initiatives, 2007). Therefore, these two varieties are dual-purpose (grain and fibre) crops. Crag and Finola, a Finnish variety that is shorter in stature, early maturing, and a with a high yield potential (Manitoba Agriculture, Food and Rural Initiatives, 2007), are predominantly grown for seed. In 2007, all four varieties were approved for cultivation, and USO 14, 31 and Crag were exempt from THC testing. Finola is under observation by Health Canada, and still requires THC testing to ensure levels are below 0.3% THC (Health Canada, 2007)

Analytical Procedures

Prior to analysis, all samples were ground initially through the use of a hand-held electric coffee mill. For all samples, % crude protein (CP; N x 6.25) was determined through the use of a LECO CNS-2000 Nitrogen Analyzer (LECO Corporation, St Joseph MI., U. S. A., Model No. 602-00-500), and % dry matter (DM) and ash were determined according to standard procedures (AOAC, 1995). The gross energy content (MJ/kg) was determined with an adiabatic bomb calorimeter (Parr Instrument Company Inc. Moline, Illinois, U. S. A.), and % neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the Ankom nylon-bag procedure. The % crude fat was determined by extracting crude fat into hexane (AOAC, 1995). The amino acid content of the samples were determined by acid hydrolysis using the AOAC Official Method 982.30 (1995). Methionine and cystine were determined by the performic acid oxidized hydrolysis procedure, and tryptophan was determined using alkaline hydrolysis. The content of total fibre, soluble and insoluble fibre were determined using the gravimetric method previously described (AOAC 1995). The contents of select non-starch polysasccharides (NSP) constituents were measured using an enzyme digestion-gas chromatography method previously described (Meng and Slominski, 2005). Phytate-bound phosphorus content of hemp protein flour samples was analyzed according to the method of Haug and Lantzsch (1983).

Protein Digestibility-Corrected Amino Acid Score (PDCAAS) Determination

In consultation with the Canadian Food Inspection Agency, the PDCAAS method was chosen as a method to establish a protein value for hemp, using casein as a control or reference protein (see Appendix 1 for email correspondence). For PDCAAS measurements, 2 hemp seed samples, 3 dehulled hemp seed products, and 3 hemp seed meals were selected. The PDCAAS was determined using the rat bioassay, as previously described (FAO/WHO 1990). Amino acid ratios for the 8 test articles and the test casein were derived by dividing the mg of each indispensable amino acid per gram of test protein by the mg of the same amino acid in a one gram portion of the FAO/WHO reference pattern. The reference pattern used was the FAO/WHO/UNU (1985) pattern of requirements for children 2 to 5 years of age (Table 2). Amino acid scores were determined by selecting the value of the amino acid with the lowest ratio (first limiting amino acid).

True protein digestibility was determined using the AOAC Official Method 991.29 rat bioassay (1990), using casein as a reference standard, and correcting for endogenous protein losses using a protein-free diet. Hemp seed and hemp nut samples were defatted prior to analysis. All test articles were ground to pass through a 2 mm screen prior to preparation of the test diets. Diets were formulated to contain 10% protein, from the test hemp article, 10% total fat (total of residual hemp oil and corn oil), and 5% cellulose, with the remaining energy derived from corn starch. Vitamins and minerals (AIN-93 formulations; Harlan Teklad, Madison, WI) were added to diets to meet the micronutrient requirements of laboratory rats. Male weanling laboratory rats (n=6 per treatment; initial weight 70 grams) were individually housed in suspended wire-bottomed cages, with absorbent paper placed underneath. Water was available for *ad libitum* consumption. Feed was restricted to a maximum of 15 g/day over a four day acclimation period followed by a 5 day balance period, during which daily feed intake was calculated. Total fecal output was collected during the balance period, air-dried, and retained by dry matter and nitrogen determination. True protein digestibility (TPD%) was calculated as follows:

TPD% = ((Nitrogen Intake – (Fecal Nitrogen Loss – Metabolic Nitrogen Loss))/Nitrogen Intake) x 100

where **Nitrogen Intake** and **Fecal Nitrogen Loss** represent the product of food intake or fecal weights and their respective nitrogen values. The value for **Metabolic Nitrogen Loss** was determined as the amount of fecal nitrogen produced per gram of diet consumed by rats consuming a protein-free diet. As an additional marker of protein quality, rat weights were recorded throughout the acclimation and balance periods, and feed conversion efficiency, measured as the amount of gain per unit of feed, was calculated and expressed as a percentage of that afforded by the rats consuming the casein reference diet. This value is consistent with the protein efficiency ratio (PER).

Statistical Treatment of Data

Means, standard deviations, and percent coefficients of variation were calculated for chemical constituents within a hemp product group. Due to the small sample size re: year, cultivar, and growing condition, no attempt was made to analyze the variance associated with these parameters. Regression analysis was performed for proximate variables and digestibility data, using SigmaPlot 2000 (SPSS Inc.).

RESULTS AND DISCUSSION

Proximate Analyses of Hemp Seed and Hemp Seed Products

In order to develop meaningful nutritional messages for foods, knowledge of the nutrient composition of the food in question is critical. At a minimum, an understanding of the proximate composition, that it, the content of the major macronutrients, is critical for assessing the quality of a food. The content of proximate components for the four hemp products tested are given in Table 3, and summarized in Figure 1. Intact hemp seed contains approximately 24% crude protein, 30% crude fat, and 32% neutral detergent fibre (a reflection of total fibre content), 5% ash (a reflection of the total mineral content), with the remainder as either water or nitrogen-free extractives (sugars, etc…). These values are in general agreement with data published previously (Callaway, 2004; Silversides and Lefrançois, 2005). Silversides and Lefrançois (2005) reported crude protein, crude lipid, and gross energy values of 24.9%, 33.2%, and 24.9 MJ/kg, respectively in a sample of Unika-b hemp, a variety grown in Eastern Canada for fibre. The current study did not have a sample of this hemp variety for comparison purposes, but the observed values were consistent with the mean values obtained. Callaway (2004) reported a crude protein and fat content of 24.8 and 35.5%, respectively, for Finola™ hemp seed. In the current study, samples HS5a, 5b, and 6 represented Finola™ varieties, and the mean protein and oil content of these three samples were 23.0 and 30.4%, respectively. Therefore, while crude protein values were similar to previously published values, total oil content appeared to be lower in the current trial. Since numerous conditions, including geography, climatic conditions, and local agronomic factors, likely impacted the hemp studied in the current report vs. previously reported data, it is not possible to draw extensive conclusions on the factors that influence the proximate composition of hemp seed. In general, the coefficients of variation (% CV) for protein, fat, and fibre were less than 10% for the hemp seed samples tested in the current report. As the samples were drawn from 4 distinct hemp cultivars, this low level of variation suggests future efforts to select for enhancements in specific components (ie: oil) may be limited due to low phenotypic variability.

Hemp nuts, as defined in the current report, are hemp seeds that have been de-hulled. As the hemp hull fraction contains a significant fibre (NDF) fraction (Table 3), removal of the dilution effect of the hull from the hemp seed should yield a product that is enriched in fat and protein. Indeed, that is the case: The hemp nut fraction contains 1.5 times (by weight) the amount of fat and protein than the parent hemp seed (Table 3). The higher percentage of oil in the hemp nut product is also represented in the gross energy value of the product (27.74 MJ/kg) vs. the parent seed (24.18 MJ/kg), as lipid is a more energy dense than carbohydrate (fibre, starch, sugars) fraction that has been removed during de-hulling. In general, the percentage coefficients of variation for the hemp nut products are below 12%, with the exception of the ADF and NDF fractions. The latter results are explained by one sample (HN6) having a substantially higher NDF and ADF value and a corresponding decrease in both lipid and protein content, relative to the mean values. It is likely that this sample retained more hull fraction during processing than the other 5 samples and serves to highlight the importance of processing in contributing to potential variation in the nutrient profile of hemp nuts.

The production of hemp protein flour primarily consists of the removal of the oil fraction through an expeller-based process. Based on this process, the removal of hemp oil alone should theoretically lead to: a) increase in the content of other proximate components and b) a decrease in the gross energy content, due to removal of the dilution effect of the oil. Indeed, this was the case. The mean crude protein value for the hemp protein flours was

40.68%, 1.7 times higher than the corresponding value for hemp seed (Table 3). The crude fat content was reduced from 30.38% in the intact seeds, but the residual oil content was significant, at 10.17% crude fat in the flour. The latter observation reflects the fact that the removal of oil from hemp seeds is based on expeller-based extraction processes. As such, the efficiency of oil extraction can vary depending on the expeller processes used, and the extraction efficiency is generally lower than would be expected through solvent extraction processes. Silversides and Lefrançois (2005) reported crude protein, crude lipid, and gross energy values of 30.7%, 16.4% and 21.2 MJ/kg in a sample of hemp seed meal. Their results suggest that the sample of hemp seed meal that they obtained had higher residual crude fat content. Additionally, post-expeller processing may influence the final composition of the hemp protein flours. These processes could include further grinding, followed by sifting or air-classification of the flour and/or the reintroduction of hemp seed components into the flour. This level of details was not available for the current analyses, but an examination of the percentage coefficients of variation do support a higher level of variation in the nutrient components based on the processes used to produce the hemp protein flours. In general, the %CVs were higher than 20% for protein, fat, and fibre. Since the variability observed in the original hemp seed was less than 10% for the key proximate components, the current data provide strong evidence that processing effects have a substantial influence on the overall nutrient profile of the hemp protein flours.

As a means of positioning hemp seed and hemp seed products relative to other plant-based foods, data for the main nutrients water, fat, protein, total carbohydrate (determined by difference) and ash are presented in Table 4. In general, whole hemp seed tends to have lower fat and higher total carbohydrates as compared to other seeds and nuts, while the protein values tend to be consistent on a weight basis. The total carbohydrate values are determined by difference (ie: 100 - % moisture - % protein - % fat - % ash), and therefore represent the sum of fibre, starch and sugar values. On the basis of the NDF values obtained in the current study, 92% of the total carbohydrate fraction of hemp seed is represented by fibre. For seeds, this value is equal to 22% for pumpkin seeds, 56% for sunflower seeds, and 82% for sesame seeds. For nuts, this value ranges from 11% (cashews) to 69% (pecan, walnut). Therefore, relative to other seeds and nuts, whole hemp seed has a higher percentage of the total carbohydrate as dietary fibre. For hemp nuts, approximately 100% of the total carbohydrate fraction is accounted for by the NDF fraction. This may lead to marketing opportunities to position hemp seed and hemp nuts relative to their balance of protein, fat, fibre and ash.

While not addressed in the current study, a potential area for future consideration is the examination of the relative glycemic value of hemp seed and hemp seed products, due to the low percentage of non-fibre carbohydrates present. Glycemic index values provide an indication of the impact that a given food may have on raising post-prandial blood glucose and insulin values. Higher glycemic index foods produce larger fluctuations in blood glucose, and thus may confer a greater risk to individuals for developing diabetes and other co-morbidities (Ludwig, 2007). Due to the high proportion of total carbohydrate present as dietary fibre, hemp seed may therefore be a low glycemic index food, but this remains to be tested. The composition of the non-starch polysaccharides, a reflection of the dietary fibre component, of hemp products is listed in Table 5. Non-starch polysaccharides are compounds consisting of many sugar molecules joined together to form a macromolecule. They differ from the starches in that starch molecules are polymers of glucose, while the NSP molecules can consist of other sugars. An examination of Table 5 provides evidence that the primary sugars in hemp seed are xylose and glucose, each contributing roughly to 40% of the total NSP fraction by weight. The removal of the hull fraction dramatically reduces the xylose and total NSP content of hemp products, indicating a high proportion of the xylose units are present in the hull fraction. Due to the fact that certain NSP fractions, including xylans, can lead to the increase in viscosity of aqueous solutions of plant products (Mathlouthi et al., 2002) and, as a result, reduce the efficiency of absorption of certain nutrients, it is important to characterize the nutrient digestibility of hemp products, especially those containing a significant hull fraction. While generally considered to an anti-nutritive factor for feeding livestock, due to the fact that increased intestinal viscosity reduces the efficiency of nutrient absorption – a key concern for livestock producers, there is a different school of thought in relation to human nutrition. Increased gut viscosity in humans is linked to decreased post-prandial glycemic response in humans, and may therefore reduce risk factors associated with type-2 diabetes and cardiovascular disease (Jenkins et al., 2000). While beyond the scope of the current study, further research is warranted to determine if hemp products, especially those containing a significant hull fraction, would provide beneficial effects for humans in relation to the glycemic response.

One additional area for consideration relates to the ash content of the hemp products relative to the nut products: Hemp, in particular the hemp nuts, have 2 to 3 times the ash content of other nuts (Table 4), a reflection of the total mineral content. Hemp seed and hemp nuts may therefore serve as potential rich sources of minerals and trace elements. While this is the subject of a separate study conducted by the Canadian Hemp Trade Alliance, additional information on the availability of the mineral fraction can be gleaned from the examination of the fraction of phytate P that is contained within the hemp products. Phosphorus in most seeds is bound to inositol in the form of phytic acid, a storage form of phosphorus. While important as a reservoir of phosphorus for germinating seeds, the phosphorus bound up in phytate is not generally available to animals, due to an inability to breakdown the phytate molecule. The ratio of total phosphorus to phytate phosphorus provides an initial assessment of the total phytate content of the product and an assessment of phosphorus availability. The average total phytate P content of hemp protein flours (average of 8 samples) was 1.12 +/- 0.28 g of phytate phosphorus per 100 grams of hemp protein flour. Calculation of the total phosphorus content of hemp protein flours (from the data of Callaway, 2004) yields a value of 1.62 g of total phosphorus per 100 grams of hemp protein flour. Therefore, the total available phosphorus content of hemp protein flours is approximately 31% (ie: (1.62-1.12)/1.62). In relation to other food proteins, phosphorus availability (for swine; NRC, 1998) ranges from a low of 3% for sunflower meal, 14% for grain corn, 21% for canola meal, 23% for soybean meal, 30% for barley and 50% for wheat. Therefore, hemp protein flours are positioned towards the top end of the range of availabilities of total phosphorus for the major cereal grains and oil seed meals. The data do provide evidence of the presence of significant phytate fractions within hemp seed products and further research is warranted to determine what, if any, impact this fraction will have on the availability of other trace elements, since phytic acid is known to bind to other nutrients.

With respect to hemp protein flour, a comparison to other seed meals, namely soy and canola, reveals that hemp protein flour is higher in crude fat, and comparable in absolute quantities of protein and carbohydrate. The higher fat content in hemp protein flour reflects the extensive use of solvent extraction in the manufacture of soy and canola oil, which is a more efficient process for removing oil from oil seeds. The higher residual fat content will translate to a higher gross energy content for the hemp protein flour, but the availability of this energy would need to be determined with *in vivo* trials in either humans or animals. This concept of nutrient availability will be addressed in the next section detailing the research conducted to position the protein digestibility of the various hemp products.

Protein Quality of Hemp Seed and Hemp Seed Products

In general, the nutritional quality of a protein can be defined by 1) the relative contribution that the amino acids contained in the protein make to an individual's amino acid requirement and 2) the digestibility of the protein. With respect to the amino acid profile of hemp seed and hemp seed products, these values are given in Table 6. Hemp seed and hemp seed products contain all of the indispensable amino acids required by humans. With respect to the amino acid supply relative to human amino acid requirements, the amino acid scores are presented in Table 7. The amino acid score of a protein reflects the extent to which a dietary protein meets meets the individual amino acid needs of an individual. Scores of 1.0 or greater for individual amino acids indicate that, for the specific amino acid in question, it is not limiting relative to requirements. When scores are less than 1.0, the provision of the dietary protein source will yield an intake for a specific amino acid below its requirement level. The lowest score is taken as the amino acid score for the entire protein source, irrespective of the relative contributions of other amino acids. On the basis of the amino acid composition of hemp seed and hemp protein products, lysine is the first limiting amino acid in all hemp protein sources tested (Table 7), and the amino acid scores for hemp seed, hemp nuts, hemp protein flour and hemp hulls are 0.62, 0.61, 0.58, and 0.50, respectively. Depending on the source, leucine or tryptophan will be the second or third limiting amino acid. All other amino acids yield scores greater than 1.0. The shift in amino acid scores for the hemp products likely reflects a relatively higher proportion of proteins with lower lysine content in the hull fraction.

The data presented in Figure 2 positions hemp protein sources relative to other dietary proteins, on the basis of amino acid score. In general, animal protein sources provide the highest amino acid scores. This is generally expected due to the role that these dietary proteins play in animal development. Relative to other vegetable-based protein sources, the limitation in the lysine content of hemp protein positions this protein source in the same range as the main cereal grains. Oil seed meals, due to their higher proportion of lysine, yield higher relative amino acid scores. In comparison to other nut proteins, the hemp proteins yield, on average, higher amino acid scores (Figure 3). As such, hemp proteins have a competitive advantage to other nut proteins with respect to the amino acid score – one key factor contributing to the quality of dietary proteins. However, as mentioned previously, the amino acid score provides only one measure of protein quality. In order to better quantify the quality of a dietary protein, allowances should be made for how well the protein is digested and utilized by the body. This is the concept behind the protein digestibility-corrected amino acid score (PDCAAS).

The data for the digestibility of select hemp protein sources are provided in Table 8, along with the reference protein casein. The use of a reference protein, such as the high quality protein casein, provides a "benchmark" for comparisons against other studies assessing protein quality in foods. In the current study, the digestibility of the casein was determined to be 97.6%. (ie: 97.6% of the protein contained in the casein disappeared along the digestive tract of the rat and is assumed to be utilized to meet the protein needs of the animal). The digestibility of the protein in the two intact hemp seed samples studied averaged 85.2%. A similar protein digestibility was observed for the hemp protein flour samples (86.7%). This result provides evidence that the process used in expelling the oil from the hemp seed does not lead to a reduction in the digestibility of the protein contained in the seed. Heat-damaged proteins have been shown to have a lower protein digestibility (Sarwar, 1997). Since oil expellers use high pressure, significant heat can be produced during the process, but the current data provide evidence that this heat is insufficient to reduce the digestibility of the protein contained in the hemp protein flour. Additionally, the net protein efficiency ratio (PER) presented in Table 8, is the same for hemp seeds and the hemp protein flour. The PER calculated in the current study provides a different measure of the quality of the protein and reflects the ability of the test subject (ie: growing rat) to deposit body protein. The values obtained for protein digestibility and PER are consistent for both the hemp seed and the hemp protein flour.

While a detailed description of the factors affecting within sample variability in protein digestibility is not possible, due to the low numbers of samples tested, it is interesting to note that the one hemp protein flour that had the highest protein digestibility value (HPF4 = 92.1%) also had approximately half of the total NDF (21.81 vs 38.08 & 38.57%) as the other two samples (HPF1 & HPF8). Further evidence that NDF content influences the digestibility of the protein fraction is provided by the data for the hemp nut protein. Removal of the hull fraction from the hemp seed leads to an average increase in protein digestibility from 85.2% to 94.9%. Within the hemp nut samples, the one sample with the lowest protein digestibility (HN6 = 90.8%) had the highest content of NDF (18.12%). Regression of the protein digestibility values against the percent NDF of the samples (corrected to a fat-free basis to account for the fact that samples were defatted prior to feeding as per the PDCAAS protocol) provided strong evidence of the digestibility depressing effect of the hemp hull (Figure 4). The exact nature of the depressing effect of the NDF fraction on protein digestibility is not clear, but may be related to the presence of key NSPs, such as xylans (Table 5), which can increase gut viscosity and depress nutrient availability (Meng and Slominski, 2005). In general, the range of true protein digestibility values observed falls within the values observed for other high quality food proteins (FAO/WHO, 1990; Sarwar, 1997). Protein digestibility values below 80% are often related to heat damaged proteins or other processing effects (Sarwar, 1997). It is important to note that, for the hemp samples studied in the current study, no additional processing steps were involved prior to analysis. Caution must be used in extending protein digestibility values to hemp-containing foods that have been subjected to high heat or oxidizing conditions during processing.

The product of the true protein digestibility values and the amino acid score is the PDCAAS (Table 8). In general, the amino acid score has the largest impact on the PDCAAS value, due to the high values observed for protein digestibility. Therefore, unless protein digestibility is substantially depressed due to dramatic increases in hull fraction (ie: added hulls or breeding efforts) or further processing (high heat or oxidizing conditions), the PDCAAS value of hemp protein products will continue to remain in the 0.5 to 0.6 range due to the limitation in lysine content. Future efforts to breed for enhanced lysine content may be warranted if the value of the hemp protein component for human consumption dictates future market development for this crop.

In comparison to other protein foods, the PDCAAS value for hemp protein sources is positioned in the same range as the major pulse protein sources (lentils, pinto beans), and above cereal grain products, such as whole wheat. This is especially true for the hemp nut protein. In comparison to another nut protein source, all hemp protein sources are positioned higher in terms of PDCAAS than almonds (Table 9). While PDCAAS data is not generally available for all nut proteins, examination of the data in Figure 3 for the amino acid score values and assuming a generalized protein digestibility value of 0.85, the PDCAAS for hemp nut proteins should exceed that for all other nuts. The PDCAAS value for hemp seed and hemp protein flour ranks very high relative to other nut sources, potentially exceeded only by the cashew (Figure 3).

The PDCAAS method is internationally recognized as an official method for the measurement of protein quality. In Canada, while the official method for determination of protein rating is method F0-1, which is based on the protein efficiency ratio (PER) method, the PDCAAS is considered an acceptable alternative provided a reference protein is used (See Appendix 1 for memo re: CFIA position). The use of the PDCAAS method in the current study afforded the opportunity to calculate a surrogate measure of the PER using the growth data obtained in the rat digestibility trial. The calculated PER values are presented in Table 8, along with the PDCAAS values. In order to provide further support for the use of the PDCAAS method in Canada, specifically applied to hemp protein, regression analysis of PER and PDCAAS revealed a strong relationship between these values (r^2 =0.86; Figure 5).

While the amino acid scoring method is primarily targeted towards establishing protein quality estimates for human nutrition purposes, the same concept can apply in positioning a protein for other species as well. The amino acid scores of the hemp protein sources have been calculated for the broiler chicken, laying hen, cat, dog, horse, and pig, and these are presented in Figure 6. In general, the hemp protein sources provide higher scores for other species than for the human, and this may provide opportunities to position hemp proteins for livestock or domestic animal feeding purposes. Indeed, the feeding value of hemp has been established for both the ruminant (Mustafa et al., 1999) and for the laying hen (Silversides and Lefrançois, 2005). Future opportunities in this area would require efforts to have the Canadian Food Inspection Agency recognize hemp protein sources as permissible ingredients for livestock feeds. Continued research on the safety and nutritional quality of hemp protein sources will help is establishing the necessary evidence to do so.

When establishing the official protein quality value of a protein, the bulk of the attention is paid to the first limiting amino acid, as this one will set the amino acid score and influence net protein deposition in a growing rat. From a practical standpoint, however, dietary protein sources, especially in developed countries, are rarely consumed in isolation. The practice of protein complementation, or blending protein sources such that the whole is greater than the sum of the parts, is an effective tool for correcting limitations in the amino acid supply that exist with a single protein source. In the case of hemp proteins, complementing them with protein sources higher in lysine, such as pulse crops, will improve the overall amino acid score of the blend. In addition to recognizing that limitations in amino acid supply can be overcome through protein complementation, opportunity also exists to evaluate the potential contribution of other amino acids to the diet. As depicted in Figure 7, hemp proteins contain a high amount of arginine, relative to other food proteins. Arginine serves as a dietary precursor for the formation of nitric oxide (Wu and Meininger, 2002), a potent mediator of vascular tone and, therefore, may have implications for the health of the cardiovascular system. Additionally, arginine, or nitric oxide specifically, has been linked to optimal immune function (Grimble, 2005) and to muscle repair (Archer *et al*., 2006). As more evidence accumulates linking arginine nutrition to improved health outcomes, the potential exists to position hemp proteins as an optimal source of digestible arginine.

SUMMARY

In summary, the protein from hemp seed is highly digestible in either its native form or as a protein flour. Removal of the hull fraction from the hull improves the digestibility of the protein and the corresponding PDCAAS, due to the removal of significant NDF components which limit protein digestion. Improvements in the protein quality of hemp will be geared to the lysine content of hemp proteins, as this amino acid is the limiting amino acid in current protein quality evaluation techniques. The potential exists to include lysine content in future hemp breeding programs in order to enhance the content of this amino acid in hemp proteins. In general, hemp seed and hemp protein flours provide a high fibre, high protein food that is well positioned to take advantage of multiple nutrition promotion messages. Additional opportunities for hemp protein sources may be realized through capturing new markets (ie: organic livestock production; pet food production) or through the identification of new health promoting properties (ie: arginine supply).

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TABLES

Table 1. Characteristics of the analyzed hempseed products

Sample Key	***Product***	***Variety***	***Cropping Year***	***Location***
HS1	Hempseed	USO 31	2004	Manitoba
HS2	Hempseed	USO 31	2003	Manitoba
HS3	Hempseed	USO 14	2004	?[1]
HS4	Hempseed	USO 14	2003	?
HS5a	Hempseed	Finola	2004	?
HS5b	Hempseed	Finola	2004	?
HS6	Hempseed	Finola	2003	Manitoba
HS7a	Hempseed	Crag	2004	Manitoba
HS7b	Hempseed	Crag	2004	Saskatchewan
HS8	Hempseed	Crag	2003	?
HS9	Hempseed	Crag	2003	?
HN1a	Hemp Nuts	USO 31	2004	?
HN1b	Hemp Nuts	USO 31	2004	Saskatchewan
HN2	Hemp Nuts	USO 31	2003	Manitoba
HN4	Hemp Nuts	USO 14	2003	?
HN5	Hemp Nuts	Crag	2004	Manitoba
HN6[2]	Hemp Nuts	USO-31	2004	Manitoba
HPF1	Hemp Protein Flour	USO 31	2004	?
HPF2	Hemp Protein Flour	USO 31	2003	?
HPF4	Hemp Protein Flour	USO 14	2003	?
HPF4a	Hemp Protein Flour	Finola	2004	Manitoba
HPF4b	Hemp Protein Flour	Finola	2004	Saskatchewan
HPF5a	Hemp Protein Flour	Crag	2004	Manitoba
HPF7	Hemp Protein Flour	Unknown	2004	?
HPF8	Hemp Protein Flour	Crag	2004	Saskatchewan
HPF9	Hemp Protein Flour	Finola	2004	?
HPF10	Hemp Protein Flour	Finola	2005	?
HH1	Hemp Hulls	USO-31	2004	Manitoba
HH1F	Hemp Hulls	Unknown	?	?
HH2	Hemp Hulls	Unknown	?	?

Notes: [1]? = variable unknown; [2]Includes fines

Table 2. FAO/WHO/UNU (1985) pattern of amino acid requirements for preschool chidren (2-5 years of age) used for determining the amino acid score of test proteins

Amino Acid	*Requirement Pattern (mg/g protein)*
Histidine	19
Isoleucine	28
Leucine	66
Lysine	58
Methionine + Cysteine	25
Phenylalanine + Tyrosine	63
Threonine	34
Tryptophan	11
Valine	35

Table 3. Proximate analysis measures and gross energy content of hemp seed and hemp seed products.

	DM (%)	Fat (%)	CP (%)	ADF (%)	NDF (%)	Ash (%)	GE (MJ/kg)
Hemp Seeds							
HS1	90.77	25.58	21.87	24.32	32.98	5.65	23.60
HS2	94.28	29.98	25.46	23.37	34.22	3.67	23.63
HS3	95.57	31.43	24.01	21.90	31.87	5.15	23.97
HS4	91.23	25.44	21.31	26.08	36.21	4.51	23.54
HS5a	93.70	29.52	21.93	24.96	33.21	5.17	24.51
HS5b	91.78	30.30	23.84	21.78	29.34	4.27	24.70
HS6	95.56	31.34	23.23	25.18	34.46	3.72	24.83
HS7a	95.14	31.71	27.53	21.90	27.77	5.13	24.28
HS7b	95.32	32.89	23.27	22.40	32.34	4.70	24.26
HS8	95.62	33.03	27.21	23.93	31.39	4.89	24.16
HS9	96.04	33.00	24.07	22.48	29.58	5.94	24.54
Mean	***94.09***	***30.38***	***23.97***	***23.48***	***32.12***	***4.80***	***24.18***
S.D	***1.95***	***2.69***	***2.05***	***1.51***	***2.50***	***0.72***	***4.51***
%CV	***2.07***	***8.85***	***8.56***	***6.43***	***7.77***	***15.04***	***18.64***
Hemp Nuts							
HN1a	93.65	45.94	38.54	2.59	6.65	6.87	27.74
HN1b	93.74	46.54	38.45	1.39	5.60	7.06	27.89
HN2	94.29	49.30	36.49	0.93	6.06	7.03	28.51
HN4	96.60	48.86	38.69	2.10	6.03	5.59	28.95
HN5	96.97	52.25	32.67	0.55	4.59	6.20	28.03
HN6	95.39	37.56	30.34	11.95	18.12	5.37	25.33
Mean	***95.11***	***46.74***	***35.86***	***3.25***	***7.84***	***6.36***	***27.74***
S.D	***1.44***	***5.03***	***3.55***	***4.33***	***5.08***	***0.75***	***1.26***
%CV	***1.52***	***10.76***	***9.89***	***133.09***	***64.80***	***11.79***	***4.56***
Hemp Protein Flour							
HPF1	98.83	8.88	31.49	23.06	38.08	6.78	19.96
HPF2	91.92	10.59	44.26	20.40	23.10	7.10	20.31
HPF4	93.91	15.48	44.72	14.08	21.81	7.13	21.65
HPF4a	92.33	8.77	53.28	12.35	20.92	6.82	20.23
HPF4b	94.30	9.45	47.74	16.93	26.92	6.30	20.67
HPF5a	94.85	10.47	33.09	32.00	41.46	6.78	19.63
HPF7	94.17	8.39	35.10	27.89	37.21	4.64	20.57
HPF8	95.41	11.91	33.66	23.53	38.57	6.82	21.05
HPF9	98.59	8.56	31.04	27.87	37.40	6.07	20.42
HPF10	96.58	9.16	52.46	16.74	19.04	8.68	19.59
Mean	***95.09***	***10.17***	***40.68***	***21.48***	***30.45***	***6.71***	***20.41***
S.D	***2.34***	***2.17***	***8.77***	***6.51***	***8.83***	***1.01***	***0.63***
%CV	***2.46***	***21.34***	***21.55***	***30.32***	***28.99***	***14.99***	***3.09***
Hemp Hulls							
HH1	96.96	15.75	16.32	44.88	55.72	4.08	21.44
HHIF	93.36	10.91	12.79	48.75	64.71	3.14	20.22
HH2	94.46	4.30	8.84	56.88	74.23	4.37	18.77
Mean	***94.93***	***10.32***	***12.65***	***50.17***	***64.88***	***3.87***	***20.15***
S.D	***1.84***	***5.75***	***3.74***	***6.12***	***9.26***	***0.64***	***1.34***
%CV	***1.94***	***55.70***	***29.60***	***12.21***	***14.27***	***16.61***	***6.63***

Notes: [1]dry matter content; [2]fat content determined by hexane extraction; [3]crude protein = nitrogen content x 6.25 (determine by LECO analysis); [4]ADF = acid detergent fibre; NDF = neutral detergent fibre

Table 4. Comparison of the nutrient profiles of hemp seed and hemp seed products to other seeds, seed meals, and nuts.

	Water (%)	Fat (%)	Protein[1] (%)	Total CHO[2] (%)	Ash (%)
Hemp Seeds	5.91	30.38	23.97	34.94	4.80
Hemp Nuts	4.89	46.74	35.86	6.15	6.36
Hemp Protein Flour	4.91	10.17	40.68	37.53	6.71
Hemp Hulls	5.07	10.32	12.65	68.09	3.87
Seeds					
Flax Seed[3]	6.96	42.16	18.29	28.88	3.72
Pumpkin Seed[4]	6.92	45.85	24.54	17.81	4.90
Sesame Seed[4]	4.81	54.78	20.33	15.44	4.60
Sunflower Seed[4]	5.36	49.57	22.78	18.76	3.50
Seed Meals					
Soybean Meal[3]	6.94	2.39	49.20	35.89	5.58
Canola Meal[5]	10.00	3.50	35.00	45.40	6.10
Nuts					
Almond[4]	4.70	49.42	21.22	21.67	2.99
Brazil Nut[4]	3.48	66.43	14.32	12.27	3.51
Cashew[4]	5.20	43.85	18.22	30.19	2.54
Hazelnut[4]	5.31	60.75	14.95	16.70	2.29
Macadamia[4]	1.36	75.77	7.91	13.82	1.14
Peanut[4]	6.50	49.24	25.80	16.13	2.33
Pecan[4]	3.52	71.97	9.17	13.86	1.49
Pine Nut[4]	2.28	68.37	13.69	13.08	2.59
Pistachio[4]	3.97	44.44	20.61	27.97	3.02
Walnut[4]	4.56	59.00	24.06	9.91	2.47

Notes: [1]Protein = nitrogen content x 6.25 (determine by LECO analysis); [2]Total CHO = total carbohydrate determined by difference and includes fibre component; [3]Data source from Health Canada's Canadian Nutrient File 2007 (accessed: http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index_e.html); [4]Data sourced from USDA Nutrient database (accessed: http://www.nal.usda.gov/fnic/foodcomp/search/index.html); 5Data sourced from the Canola Council of Canada (accessed: http://www.canola-council.org/meal4.html).

Table 5. Non-starch polysaccharide (NSP) composition of hempseed products (% as is basis)

Sample ID	**Rhamnose**	**Arabinose**	**Xylose**	**Mannose**	**Galactose**	**Glucose**	**Uronic Acid**	**Total NSP**
	mg/g	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*
Whole Seeds								
HS1	1.11	3.47	80.28	1.54	3.02	86.07	21.31	196.78
HS4	1.28	2.78	92.74	1.14	2.73	93.85	29.44	223.97
HS5a	1.29	3.02	84.18	1.33	2.56	92.00	22.42	206.81
HS7a	1.17	3.04	56.99	1.05	2.51	50.62	25.99	141.36
HS9	0.87	2.63	59.51	1.07	2.25	60.03	19.40	145.76
Mean	***1.14***	***2.99***	***74.74***	***1.23***	***2.61***	***76.52***	***23.71***	***182.94***
S.D.	***0.17***	***0.32***	***15.74***	***0.21***	***0.28***	***19.84***	***4.00***	***37.27***
%CV	***15.07***	***10.67***	***21.06***	***16.83***	***10.78***	***25.93***	***16.88***	***20.37***
Hemp Nuts								
HN4	0.24	1.46	1.64	0.30	1.34	2.66	5.19	12.83
HN5	0.22	1.31	1.27	0.27	1.09	2.66	6.26	13.08
Mean	*0.23*	*1.38*	*1.45*	*0.29*	*1.22*	*2.66*	*5.73*	*12.95*
S.D.	-	-	-	-	-	-	-	-
%CV	-	-	-	-	-	-	-	-

Table 6. Amino acid composition of hempseed products (% as is basis)

Whole Seeds	**ASP**	**THR**	**SER**	**GLU**	**PRO**	**GLY**	**ALA**	**CYS**	**VAL**	**MET**	**ILE**	**LEU**	**TYR**	**PHE**	**HIS**	**LYS**	**ARG**	**TRP**
HS1	2.250	0.888	1.078	3.547	0.996	0.998	0.966	0.354	1.068	0.450	0.850	1.406	0.719	0.968	0.495	0.833	1.955	0.205
HS2	2.224	1.105	1.223	3.520	0.812	0.949	0.807	0.369	0.995	0.523	0.682	1.392	0.513	0.975	0.485	0.767	2.065	0.234
HS3	2.509	0.785	1.008	3.789	1.118	1.230	1.022	0.386	1.493	0.574	0.535	1.185	0.474	0.679	0.598	0.901	2.117	0.149
HS4	2.196	0.818	1.020	3.282	0.789	0.965	0.948	0.423	0.986	0.561	0.785	1.403	0.738	0.953	0.484	0.756	2.045	0.192
HS5a	2.141	0.767	0.953	3.364	0.811	0.992	0.926	0.367	1.076	0.515	0.865	1.356	0.812	1.010	0.476	0.764	2.013	0.213
HS5b	2.340	0.931	1.228	3.904	0.925	1.111	1.037	0.386	1.080	0.473	0.876	1.602	0.732	1.047	0.556	0.840	2.378	0.216
HS6	2.400	0.798	1.119	4.001	0.824	1.102	0.996	0.397	1.184	0.563	0.934	1.556	0.764	1.111	0.671	0.835	2.529	0.277
HS7a	2.717	1.343	1.444	4.211	0.868	1.207	1.116	0.459	1.180	0.709	0.805	1.725	0.678	1.106	0.605	1.024	2.763	0.198
HS7b	2.484	1.107	1.278	3.607	0.875	0.981	0.824	0.454	1.086	0.645	0.776	1.414	0.548	0.970	0.523	0.828	2.265	0.256
HS8	2.430	1.211	1.280	3.870	0.904	1.005	0.865	0.565	1.151	0.663	0.814	1.652	0.715	1.125	0.568	0.905	2.490	0.186
HS9	2.628	1.313	1.428	4.075	0.955	1.143	0.994	0.353	1.184	0.533	0.837	1.664	0.752	1.341	0.555	0.961	2.411	0.368
Mean	**2.392**	**1.006**	**1.187**	**3.743**	**0.898**	**1.062**	**0.955**	**0.410**	**1.135**	**0.564**	**0.796**	**1.487**	**0.677**	**1.026**	**0.547**	**0.856**	**2.275**	**0.227**
S.D.	**0.184**	**0.218**	**0.166**	**0.300**	**0.098**	**0.100**	**0.094**	**0.063**	**0.138**	**0.080**	**0.108**	**0.164**	**0.112**	**0.161**	**0.062**	**0.085**	**0.259**	**0.058**
%CV	**7.676**	**21.636**	**13.968**	**8.026**	**10.882**	**9.436**	**9.851**	**15.357**	**12.167**	**14.157**	**13.576**	**11.036**	**16.586**	**15.670**	**11.301**	**9.916**	**11.394**	**25.590**
Hemp Nuts																		
HN1a	3.862	1.367	1.826	6.680	2.044	1.781	1.710	0.684	1.942	0.975	1.533	2.394	1.637	1.627	1.140	1.291	4.506	0.391
HN1b	3.787	1.302	1.758	6.541	1.434	1.597	1.545	0.729	1.739	0.987	1.458	2.200	1.158	1.622	0.969	1.310	4.479	0.424
HN2	4.063	1.413	1.899	7.210	2.104	1.729	1.644	0.656	1.946	1.095	1.518	2.331	1.156	1.644	1.016	1.218	4.743	0.447
HN4	3.839	1.255	1.691	6.267	1.352	1.595	1.418	0.670	1.944	0.967	1.556	2.315	1.439	1.598	0.965	1.214	4.207	0.423
HN5	3.317	1.153	1.488	5.489	1.081	1.361	1.323	0.567	1.524	0.849	0.870	1.836	1.030	0.940	0.882	1.310	5.312	0.327
HN6	3.100	1.129	1.513	5.170	1.725	1.573	1.477	0.568	1.589	0.749	0.829	1.740	1.246	1.166	0.833	1.213	4.044	0.270
Mean	**3.661**	**1.270**	**1.696**	**6.226**	**1.623**	**1.606**	**1.519**	**0.646**	**1.781**	**0.937**	**1.294**	**2.136**	**1.278**	**1.433**	**0.967**	**1.259**	**4.549**	**0.380**
S.D.	**0.369**	**0.113**	**0.167**	**0.766**	**0.405**	**0.146**	**0.144**	**0.065**	**0.192**	**0.121**	**0.346**	**0.279**	**0.222**	**0.303**	**0.107**	**0.049**	**0.447**	**0.068**
%CV	**10.090**	**8.934**	**9.837**	**12.302**	**24.977**	**9.113**	**9.450**	**10.131**	**10.794**	**12.888**	**26.741**	**13.047**	**17.377**	**21.150**	**11.106**	**3.882**	**9.821**	**17.914**

Key: ASP = asparagine; THR = threonine; SER = serine; GLU = glutamate/glutamine; PRO = proline; GLY = glycine; ALA = alanine; CYS = cysteine; VAL = valine; MET = methionine; ILE = isoleucine; LEU = leucine; TYR = tyrosine; PHE = phenylalanine; HIS = histidine; LYS = lysine; ARG = arginine; TRP = tryptophan.

Table 6. Amino acid composition of hempseed products (% as is basis) (Continued)

Hemp Protein Flour	**ASP**	**THR**	**SER**	**GLU**	**PRO**	**GLY**	**ALA**	**CYS**	**VAL**	**MET**	**ILE**	**LEU**	**TYR**	**PHE**	**HIS**	**LYS**	**ARG**	**TRP**
HPF1	3.037	1.089	1.353	4.761	1.329	1.273	1.051	0.620	1.515	0.532	1.306	2.213	1.206	1.673	0.784	1.034	3.404	0.255
HPF2	4.132	1.337	1.895	7.079	1.632	1.874	1.801	0.828	2.100	1.078	1.604	2.479	1.095	1.763	1.128	1.517	4.683	0.468
HPF4	3.806	1.465	1.863	6.441	1.793	1.773	1.589	0.784	1.826	0.971	1.391	2.243	0.979	1.580	0.955	1.302	3.932	0.439
HPF4a	4.795	1.736	2.252	7.980	1.951	2.135	2.054	0.929	2.375	1.319	1.746	3.160	1.458	2.079	1.227	1.756	5.368	0.546
HPF4b	4.445	1.558	2.064	7.441	1.978	2.118	2.037	0.825	2.288	1.092	1.774	2.987	1.632	2.031	1.138	1.649	4.982	0.456
HPF5a	3.080	1.080	1.339	4.689	1.411	1.172	1.591	0.553	1.995	0.746	1.385	1.816	1.113	1.202	0.887	1.028	2.928	0.274
HPF7	3.080	1.108	1.482	5.250	1.322	1.572	1.467	0.602	1.692	0.733	1.331	2.127	1.121	1.485	0.785	1.210	3.446	0.332
HPF8	3.423	1.290	1.611	5.673	1.836	1.708	1.593	0.612	1.796	0.736	1.421	2.094	1.114	1.434	0.800	1.191	3.321	0.361
HPF9	3.123	1.454	1.709	4.957	1.057	1.345	1.327	0.525	1.571	0.725	1.047	1.983	0.658	1.366	0.699	1.147	3.113	0.406
Mean	**3.658**	**1.346**	**1.730**	**6.030**	**1.590**	**1.663**	**1.612**	**0.698**	**1.906**	**0.881**	**1.445**	**2.345**	**1.153**	**1.624**	**0.934**	**1.315**	**3.909**	**0.393**
S.D.	**0.667**	**0.229**	**0.316**	**1.243**	**0.324**	**0.352**	**0.322**	**0.145**	**0.304**	**0.248**	**0.230**	**0.453**	**0.276**	**0.295**	**0.189**	**0.265**	**0.887**	**0.096**
%CV	**18.221**	**17.010**	**18.283**	**20.612**	**20.385**	**21.160**	**19.980**	**20.742**	**15.963**	**28.097**	**15.892**	**19.332**	**23.911**	**18.172**	**20.238**	**20.175**	**22.688**	**24.350**
Hemp Hulls																		
HH1	1.230	0.473	0.559	1.762	1.234	0.516	0.505	0.184	0.910	0.184	0.438	0.961	0.462	0.582	0.403	0.473	1.823	0.093
HHIF	0.927	0.391	0.450	1.267	0.538	0.490	0.495	0.234	0.579	0.300	0.493	0.750	0.421	0.580	0.241	0.345	0.708	0.061
HH2	0.535	0.219	0.240	0.526	0.310	0.221	0.212	0.113	0.301	0.052	0.236	0.431	0.325	0.435	0.105	0.162	0.275	0.019
Mean	**0.897**	**0.361**	**0.416**	**1.185**	**0.694**	**0.409**	**0.404**	**0.177**	**0.597**	**0.179**	**0.389**	**0.714**	**0.403**	**0.532**	**0.250**	**0.327**	**0.936**	**0.058**
S.D.	**0.348**	**0.129**	**0.162**	**0.622**	**0.481**	**0.163**	**0.167**	**0.061**	**0.305**	**0.124**	**0.135**	**0.267**	**0.070**	**0.085**	**0.149**	**0.156**	**0.799**	**0.037**
%CV	**38.815**	**35.839**	**38.934**	**52.530**	**69.322**	**39.903**	**41.240**	**34.452**	**51.158**	**69.452**	**34.759**	**37.408**	**17.433**	**15.900**	**59.653**	**47.749**	**85.378**	**64.526**

Key:
ASP = asparagine; THR = threonine; SER = serine; GLU = glutamate/glutamine; PRO = proline; GLY = glycine; ALA = alanine; CYS = cysteine; VAL = valine; MET = methionine; ILE = isoleucine; LEU = leucine; TYR = tyrosine; PHE = phenylalanine; HIS = histidine; LYS = lysine; ARG = arginine; TRP = tryptophan.

Table 7. Amino acid scores of hemp protein products

	HIS[2]	ILE[2]	LEU[2]	LYS[2,3]	M+C[2]	P+T[2]	THR[2]	TRP[2]	VAL[2]
Whole Seeds									
HS1	1.191	1.389	0.974	0.657	1.470	1.224	1.195	0.853	1.396
HS2	1.003	0.956	0.829	0.520	1.402	0.927	1.277	0.836	1.117
HS3	1.312	0.796	0.748	0.647	1.599	0.763	0.962	0.566	1.778
HS4	1.195	1.316	0.997	0.612	1.848	1.260	1.130	0.820	1.322
HS5a	1.141	1.409	0.937	0.601	1.609	1.319	1.029	0.883	1.402
HS5b	1.227	1.312	1.018	0.607	1.441	1.184	1.149	0.824	1.295
HS6	1.521	1.436	1.015	0.619	1.654	1.281	1.010	1.082	1.456
HS7a	1.156	1.044	0.949	0.641	1.697	1.029	1.435	0.654	1.225
HS7b	1.184	1.192	0.921	0.614	1.889	1.035	1.400	1.000	1.334
HS8	1.098	1.068	0.920	0.574	1.806	1.074	1.309	0.623	1.209
HS9	1.214	1.242	1.047	0.688	1.472	1.380	1.604	1.389	1.405
Mean	***1.204***	***1.196***	***0.941***	***0.616***	***1.626***	***1.134***	***1.227***	***0.866***	***1.358***
SD	***0.131***	***0.207***	***0.088***	***0.045***	***0.170***	***0.187***	***0.199***	***0.232***	***0.172***
Hemp Nuts									
HN1A	1.557	1.420	0.941	0.577	1.721	1.344	1.043	0.922	1.440
HN1B	1.327	1.355	0.867	0.587	1.786	1.148	0.996	1.002	1.292
HN2	1.466	1.486	0.968	0.575	1.919	1.218	1.139	1.113	1.524
HN4	1.312	1.436	0.907	0.541	1.693	1.246	0.954	0.995	1.436
HN5	1.420	0.951	0.851	0.691	1.734	0.957	1.038	0.909	1.333
HN6	1.444	0.976	0.869	0.689	1.736	1.262	1.095	0.810	1.496
Mean	***1.421***	***1.271***	***0.900***	***0.610***	***1.765***	***1.196***	***1.044***	***0.958***	***1.420***
SD	***0.091***	***0.242***	***0.047***	***0.064***	***0.081***	***0.133***	***0.066***	***0.103***	***0.091***
Hemp Protein Flour									
HPF1	1.310	1.481	1.065	0.566	1.463	1.451	1.017	0.736	1.374
HPF2	1.341	1.294	0.849	0.591	1.723	1.025	0.889	0.961	1.356
HPF4	1.124	1.111	0.760	0.502	1.570	0.908	0.964	0.893	1.166
HPF4a	1.212	1.170	0.899	0.568	1.688	1.054	0.958	0.932	1.274
HPF4b	1.255	1.327	0.948	0.595	1.606	1.218	0.960	0.868	1.369
HPF5a	1.411	1.495	0.831	0.536	1.570	1.110	0.960	0.753	1.722
HPF7	1.177	1.354	0.918	0.594	1.521	1.178	0.928	0.860	1.377
HPF8	1.251	1.508	0.943	0.610	1.602	1.202	1.127	0.975	1.525
HPF9	1.185	1.205	0.968	0.637	1.611	1.035	1.378	1.189	1.446
Mean	***1.252***	***1.327***	***0.909***	***0.578***	***1.595***	***1.131***	***1.020***	***0.907***	***1.401***
SD	***0.090***	***0.147***	***0.088***	***0.040***	***0.079***	***0.156***	***0.150***	***0.134***	***0.157***
Hemp Hulls									
HH1	1.300	0.958	0.892	0.499	0.901	1.016	0.852	0.519	1.594

Notes:

[1] Reference protein = FAO/WHO amino acid requirement pattern for school children

[2] HIS = histidine; ILE=isoleucine; LEU = leucine; LYS= lysine; M+C = methionine plus cysteine; P+T = phenylalanine plus tyrosine; THR = threonine; TRP = tryptophan; VAL = valine.

[3] Shaded area reflects limiting amino acid score = lysine

Table 8. Protein digestibility-corrected amino acid score - hemp protein products

	Protein Digestibility %	Amino Acid Score[1]	PDCAAS[2]	Net Protein Efficiency Ratio[3]
Casein	**97.6**	**1.19**	**1.00**	**1.00**
Hemp Seed				
HS3	86.2	0.57	0.49	0.62
HS7a	84.1	0.64	0.54	0.69
Mean	***85.2***	***0.60***	***0.51***	***0.65***
S.D.	-	-	-	-
%CV	-	-	-	-
Hemp Nuts				
HN4	97.5	0.54	0.53	0.76
HN5	96.2	0.69	0.66	0.73
HN6	90.8	0.69	0.63	0.76
Mean	***94.9***	***0.64***	***0.61***	***0.75***
S.D.	***3.5***	***0.09***	***0.07***	***0.02***
%CV	***3.72***	***13.44***	***11.66***	***2.10***
Hemp Protein Flour				
HPF1	84.4	0.57	0.48	0.64
HPF4	92.1	0.50	0.46	0.71
HPF8	83.5	0.61	0.51	0.64
Mean	***86.7***	***0.56***	***0.48***	***0.66***
S.D.	***4.8***	***0.05***	***0.02***	***0.04***
%CV	***5.49***	***9.73***	***4.93***	***6.09***

Notes:

[1] Lysine the first limiting amino acid, using the FAO/WHO amino acid requirement pattern for school children

[2] Protein digestibility-corrected amino acid score, calculated as the product of protein digestibility and the amino acid score

[3] Surrogate measure – Determined as the ratio of feed conversion efficiency (FCE) in rats consuming test article divided by FCE of rats consuming casein

Table 9. Protein digestibility-corrected amino acid scores of hemp protein sources in comparison to other food proteins.

Protein Source	PDCAAS
Casein	1.00
Egg white	1.00
Beef	0.92
Soy protein isolate	0.92
Chickpeas (canned)	0.71
Pea flour	0.69
Kidney beans (canned)	0.68
Hemp nuts	**0.61**
Pinto beans (canned)	0.57
Rolled oats	0.57
Lentils (canned)	0.52
Hemp seed	**0.51**
Hemp protein flour	**0.48**
Whole wheat	0.40
Almond	0.23

Notes: Data for all non-hemp protein sources derived from the Joint FAP/WHO expert consultation on protein quality evaluation (1990), with the exception of the data for Almonds (Ahrens *et al*., 2005)

FIGURES

Figure 1. Summary of the proximate analysis of hemp products

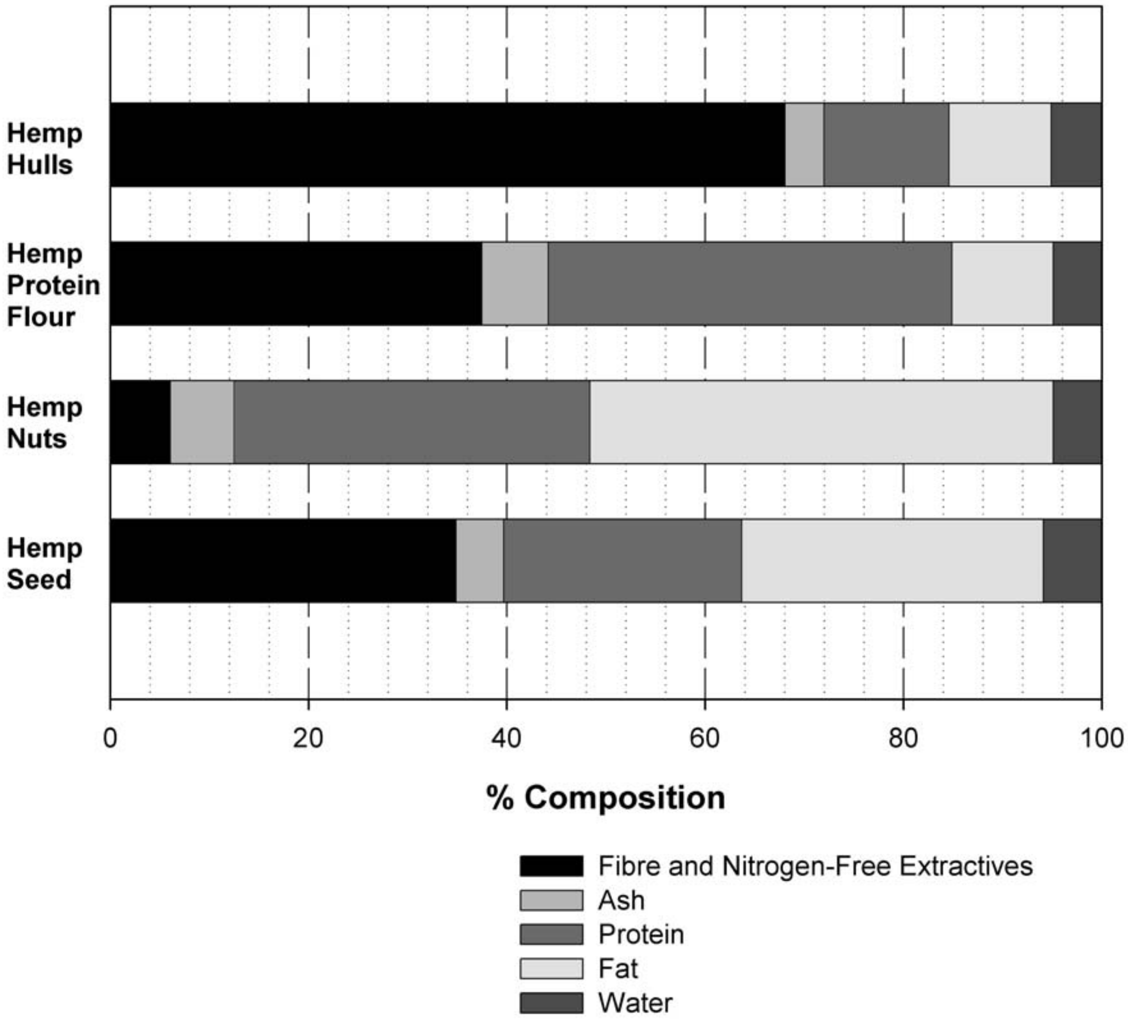
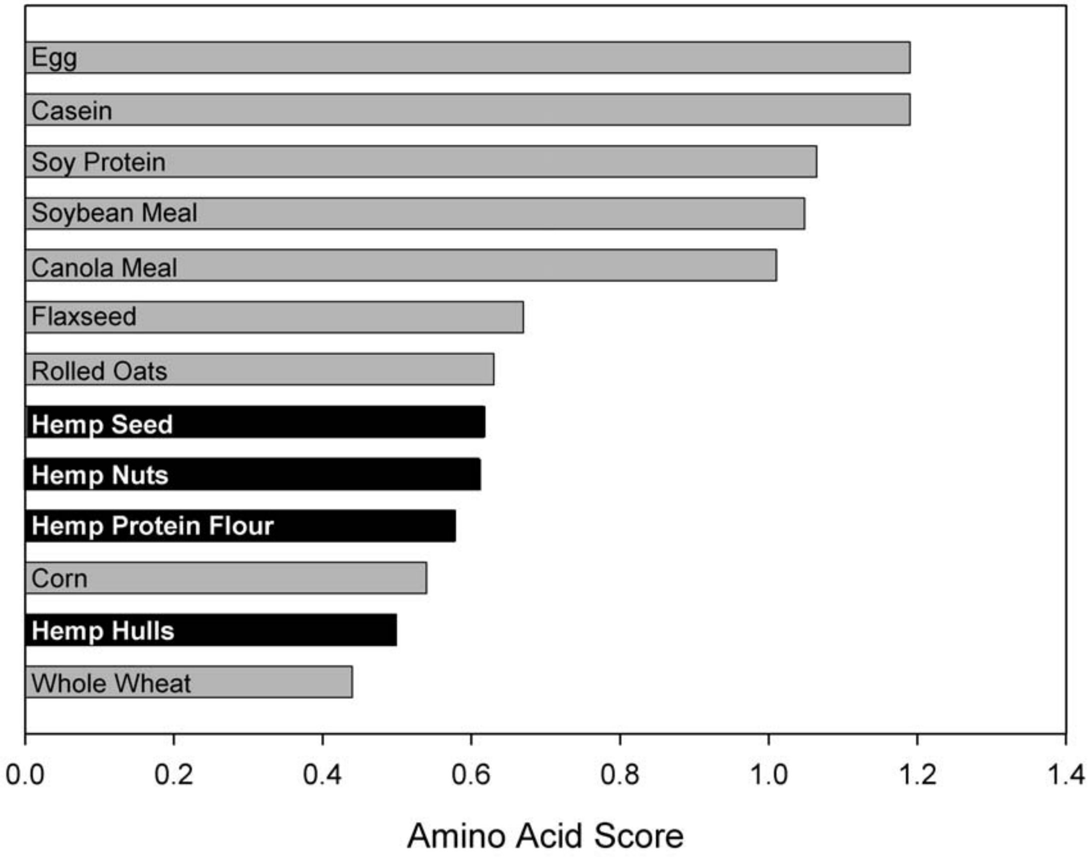
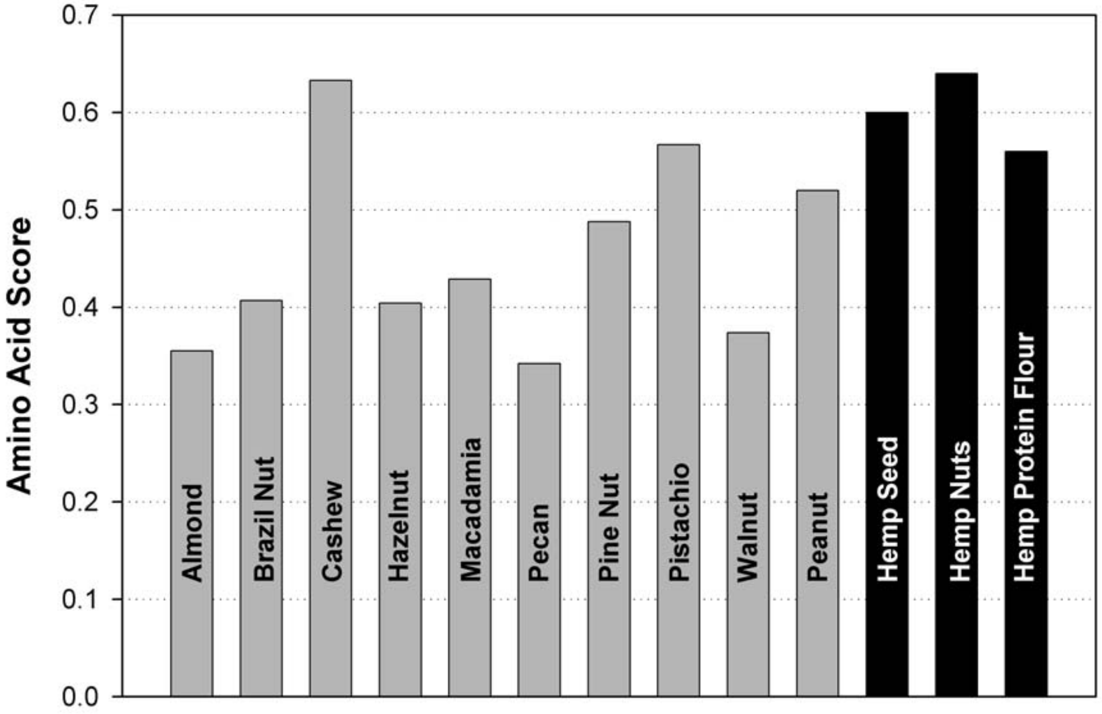


Figure 2. Amino acid scores of hemp products relative to other dietary protein sources



Notes: Amino acid scores calculated as described in "Materials and Methods" with amino acid composition of non-hemp proteins derived from the USDA Nutrient Database, Standard Reference, Release 20, 2007.
(accessed: http://www.nal.usda.gov/fnic/foodcomp/search/)

Figure 3. Amino acid scores of hemp products relative to other nut sources



Notes: Data for other nut sources derived from Venkatachalam and Sathe (2006).

Figure 4. Relationship between protein digestibility and the NDF content (corrected to a fat-free basis) of hemp protein samples

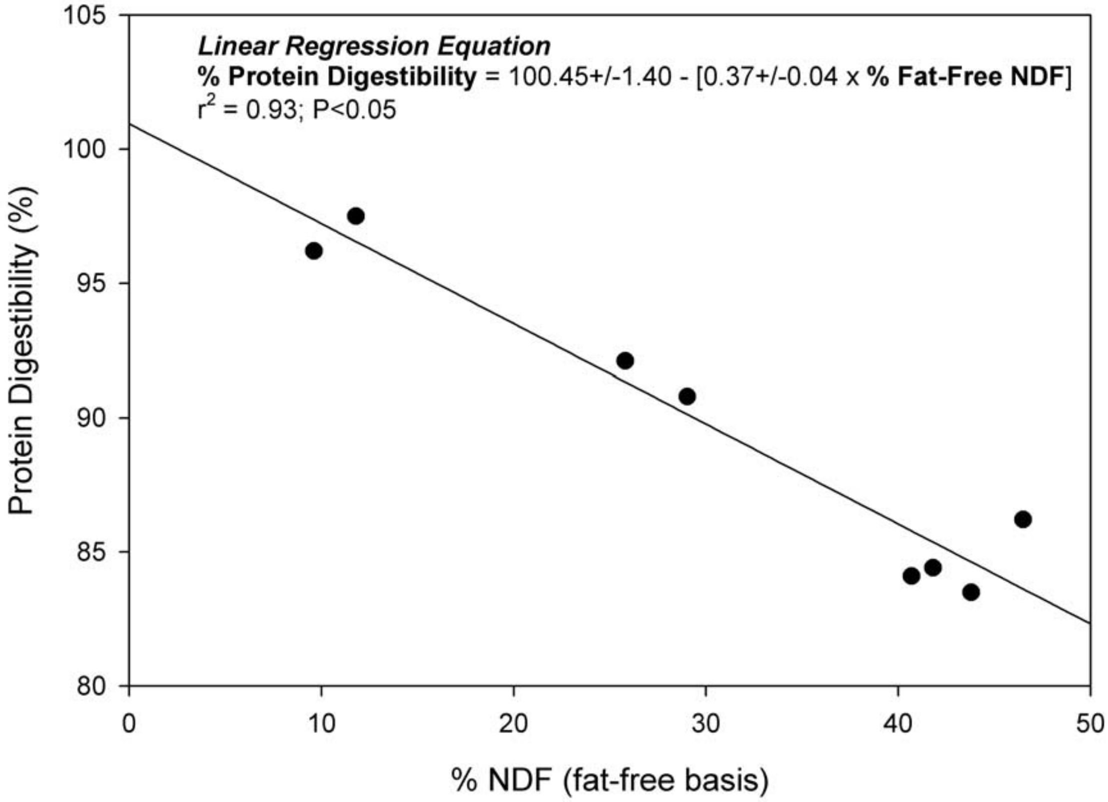


Figure 5. Relationship between PER and PDCAAS for hemp protein samples

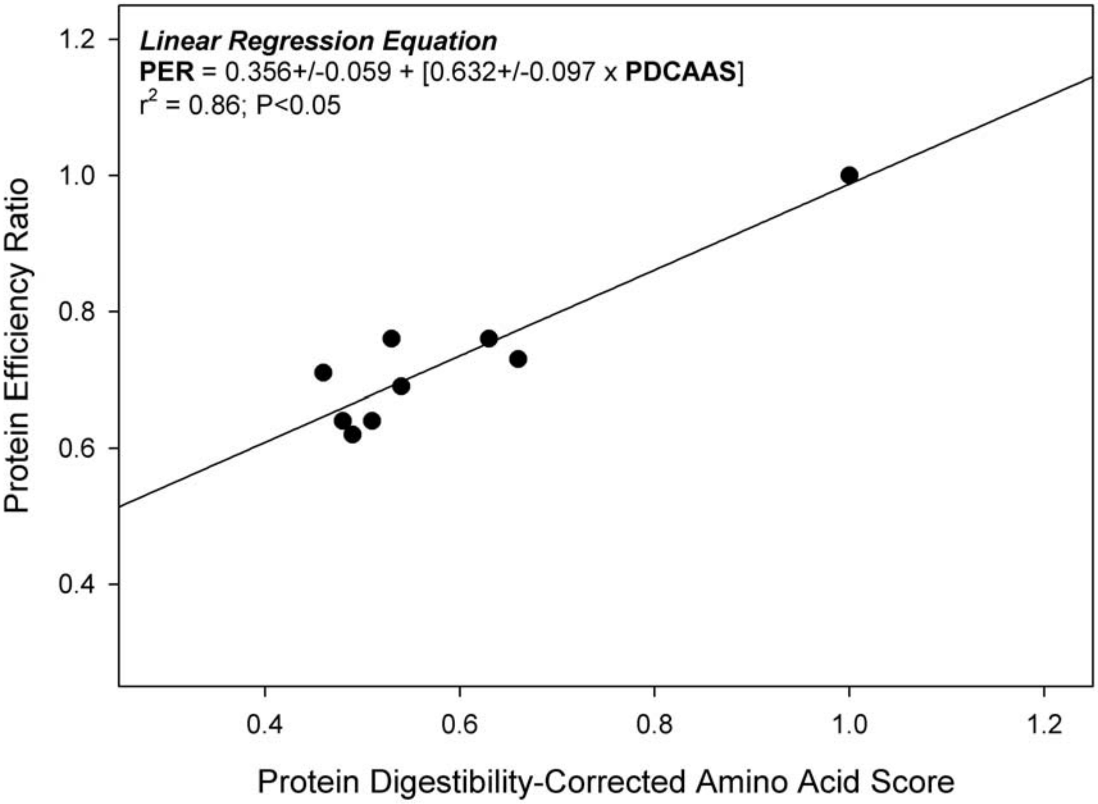
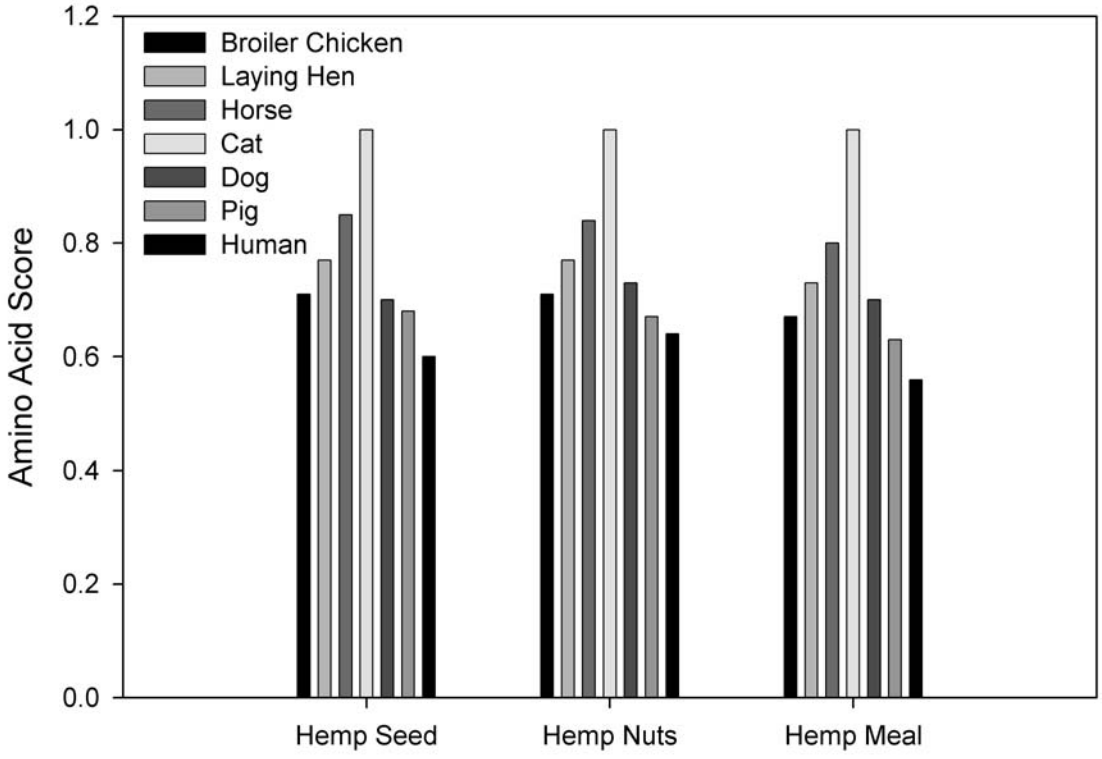
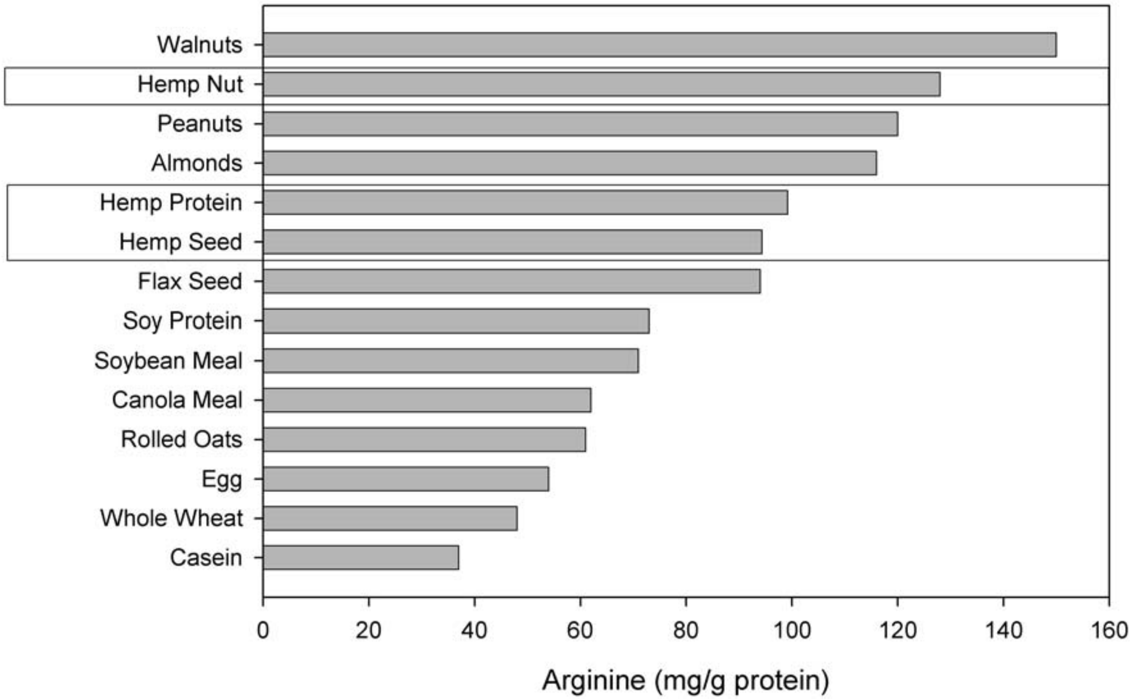


Figure 6. Amino acid scores (not corrected for protein digestibility) of hemp-based protein sources for different species



Notes: Data derived from 1) Nutrient Requirements of Poultry, NRC 1994; 2) Nutrient requirements of Horses, NRC 1989; 3) Nutrient Requirments of Cats, NRC 1986; 4) Nutrient Requirements of Dogs, NRC, 1985; 5) Nutrient Requirements of Swine, NRC 1998; 6) FAO/WHO/UNU Expert Consultation on Protein Quality Evaluation, 1989.

Figure 7. Arginine content of hemp-based protein products relative to other protein sources.



Notes: Arginine content of other proteins derived from USDA Nutrient Database Standard Reference, Release 20, 2007.
(accessed: http://www.nal.usda.gov/fnic/foodcomp/search/)

APPENDICES

Appendix 1

The following is an email received by J.D. House on Tuesday, May 18, 2004 in response to a question related to the evaluation of protein quality in Canada.

Hello Jim,

I am providing you with a copy of a response drafted by one of our program officers with respect to similar questions received by the CFIA. I trust that this will outline the CFIA position.

The official method F0-1, Determination of Protein Rating, (the PER rat study) is the method that must be used to support any protein claim.

Having said that, our stance has been with respect to the former regulations, if a manufacturer does not have a PER* for the product being sold but has an alternative way, such as the PDCAAS (the protein digestibility-corrected amino acid score) (FAO/WHO, Report of a Joint FAO/WHO Expert Consultation on Protein Quality Evaluation, 1990), to demonstrate the quality of their protein relative to casein, this information might be enough to make them confident about an estimated Protein Rating and thus about the claim they are making. It would be advisable for the manufacturer to keep on file the information and references used to make that determination. If there were any doubts** raised about the claim, however, the Official Method would have to be the one used to support the claim.

I am obtaining confirmation from my Headquarters that we will continue to take this stance with the new regulations.

I'm told that the FAO/WHO PDCASS method can be obtained at http://www.fao.org/icatalog/search/dett.asp?aries_id=1845 .

Hope this helps,

(*A manufacturer may be able to use PERs found in published literature instead of having to determine the PER of their specific product experimentally. If the product is an enzymatically-hydrolysed protein and there is no destruction of amino acids, then the PER may be assumed to be that of the intact protein from which it is derived. It is useful to check that the amino acid profile given for the product reasonably matches that of the source protein. Note that the numbers will not necessarily match perfectly - some variability is permissible - but if large differences exist, the Nutrition Evaluation Division (NED) of Health Canada should be consulted.)

(**e.g., where the protein rating just barely met the minimum level required for the protein claim and/or the protein source used in the food was uncommon (e.g., an uncommon plant material) or otherwise questionable (e.g., a hydrolyzed protein), then Health Canada could be asked to review the manufacturer's information supporting the protein claim to determine whether further investigation into the matter is warranted. This request to Health Canada should be made through the CFIA.)

Stan Bacler
(613) 221-7080 | sbacler@inspection.gc.ca | Facsimile / Télécopieur : (613) 228-6656
National Manager, Food Chemistry Laboratory Program, Canadian Food Inspection Agency
Gestionaire national du programme des laboratoires en chimie alimentaire, Agence canadienne d'inspection des aliments
Room 1052, 159 Cleopatra Drive | Pièce 1052, 159, promenade Cleopatra
Ottawa ON K1A 0Y9
Gouvernement du Canada | Government of Canada
www.inspection.gc.ca

>>> Jim House <J_House@umanitoba.ca> May 18, 2004 >>>
Hello:

I was given your name by Peter Fischer at Health Canada. I was wondering if you could let me know what the officical CFIA method is for the determination of Protein Quality. As far as I can tell from the website, the Protein Rating system is still in use, which is based on the Protein Efficiency Ratio. Is this still the case, as FAO and FDA seemed to have moved to the Protein Digestibility Corrected Amino Acid Score method?

I would appreciate any guidance that you could give me in this matter.

Regards,

Jim House

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James D. House, B.Sc.(Agr), Ph.D., P.Ag.,
Associate Professor,
Department of Animal Science,
University of Manitoba,
Winnipeg, MB, R3T 2N2,
CANADA.
Phone: 01-204-474-9523
FAX: 01-204-474-7628
e-mail: j_house@umanitoba.ca