TOTAL NITROGEN VS AMINO-ACID PROFILE AS INDICATOR OF PROTEIN CONTENT OF BEEF

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ABSTRACT

In most cited food composition studies and tables, the proximate system measures protein as total nitrogen (N) (determined by Kjeldahl or Dumas method) multiplied by a specific factor. A factor of 6.25 is used for determining total protein from total N (Jones, Munsey & Walker, 1942). Although more expensive, it is considered more accurate to base protein content of foods on amino acid data (Greenfield & Southgate, 2003).

A study on the nutrient composition of beef analyzed the full amino-acid profile of fifteen retail cuts from three age groups and six fat codes, as well as determined total nitrogen content to determine proximate protein composition. For all cuts, the correlation coefficient of total amino acids to protein (N x 6.25) was 0.635. This indicates a poor correlation for predicting actual protein content (as determined by total amino acid count), based on the nitrogen factor of 6.25. On average, the sum of amino acids per cut amounted to 91% of total determined protein (N x 6.25) for the same cut.

KEY WORDS: Nitrogen, amino acid, protein content, beef, South Africa

HIGHLIGHTS

- The importance and bioavailability of protein in human nutrition has once again been emphasized at the 1st International Symposium on Dietary Protein for Human Health and the FAO Expert Consultation on Protein in March 2011, Auckland, New Zealand.
- Amino acid count is a better determinant for total protein content, compared to the less expensive method of determining total nitrogen, multiplied by a specific factor.

- In the current study, complete amino acid profile of beef (15 cuts, over three age groups and 6 fatness classes) amounted to 91% on average of protein based on total Nitrogen content (in weight)
- Results found are in line with international results that the Jones factor of 6.25 for meat and fish might overestimate protein content based on total nitrogen.

1. INTRODUCTION

The importance and bioavailability of protein in human nutrition has recently been highlighted at the 1st International Symposium on Dietary Protein for Human Health and the subsequent Food and Agricultural Organization of the United Nations (FAO) Expert Consultation on Protein in March 2011, Auckland, New Zealand. In order to meet the protein and amino acid requirements, more information about requirements and the capacity of different foods to meet these requirements, are needed (Eliot, Beach & Robinson, 1943; Fuller, 2011).

For protein, food composition studies and tables use the proximate system of measuring protein as total nitrogen (N) (determined by Kjeldahl or Dumas method) multiplied by a specific factor. This factor has originally been 6.25 based on the assumption that all proteins contained 16% nitrogen. However, it has been known for some time that plant proteins (and gelatin) contain more nitrogen, and thus require a lower factor (Sosulski & Imafidon, 1990). Different factors originally determined by Jones *et al.* (1942), are currently used to calculate proximate protein amounts based on nitrogen content in different foods. These factors range from 6.37 for human milk, to as low as 5.55 for gelatin and 5.18 for almonds. The nitrogen factor for meat and fish is 6.25 (Greenfield & Southgate, 2003).

It is considered more scientifically correct, although more expensive, to base estimates of protein content on amino acid content, as during digestion of protein-containing foods, amino acids are as the building blocks of protein absorbed and utilized in the human body. However, in order to ensure the accuracy of using amino acid content in determining total protein, concerns such as free amino acids and soundness of analytical data, need to be taken into account.

A study was commissioned in South Africa during which 15 cuts from beef carcasses of three age groups and six fatness classes were analyzed for total nitrogen as well as complete amino acid profiles. The study conducted on the complete nutrient composition of South African beef analyzed the full amino-acid profile (HPLC) of specific cuts, as well as determined total nitrogen content to calculate proximate protein composition via the Dumas method. As age of the animal, the fatness of the animal and a particular cut may have an effect; it was decided to include all these variables in the study.

2. METHODOLOGY

2.1. Sampling

Beef carcasses, weighing between 190kg and 240kg, were selected to represent the South African commercial market and the national carcass classification system. This system classifies carcasses based on age and carcass fatness, regardless of genotype (Agricultural Product Standards Act, 1990 (Act No.119 of 1990)). The three age groups selected were from class A (younger than 18 months and no permanent incisors have erupted), class AB (18 to 24 months, with one to two permanent incisors), and class C (older than 36 months with seven or more permanent incisors). Carcasses representing the fatness spectrum (six fat classes) within each age group were selected, following visual assessment of carcass fat content and fat distribution by a trained official. The criteria for each fat class are presented in Table 1.

Table 1. Description, percentage subcutaneous fat and fat thickness for the respective fat classes in the South African classification of beef carcasses.

Fat class	Description	Subcutaneous fat (%)	Fat thickness (mm)
0	No fat	<1	0
1	Very lean	1–3.6	<1
2	Lean	3.6–5.6	1–3
3	Medium	5.6–7.6	>3 and ⩽5
4	Fat	7.6–9.6	>5 and ⩽7
5	Overfat	9.6–11.7	>7 and ⩽10
6	Excessively fat	>11.7	>10

Each side of every carcass was subdivided into 15 wholesale cuts (neck, fore shin, shoulder, chuck, brisket, prime rib, wing rib, loin, thin flank, rump, fillet, thick flank, silverside, topside and hind shin). The 15 cuts are presented in Figure 1. Each cut was accurately weighed and dissected (at 10°C ambient

temperature) into subcutaneous fat, meat (muscle and inter- and intramuscular fat) and bone, in order to determine the physical composition of each cut and, by summation, the entire carcass. The cuts from the left sides of the carcasses were used for raw sampling, while the cuts from the right sides of the carcasses were cooked prior to dissection.

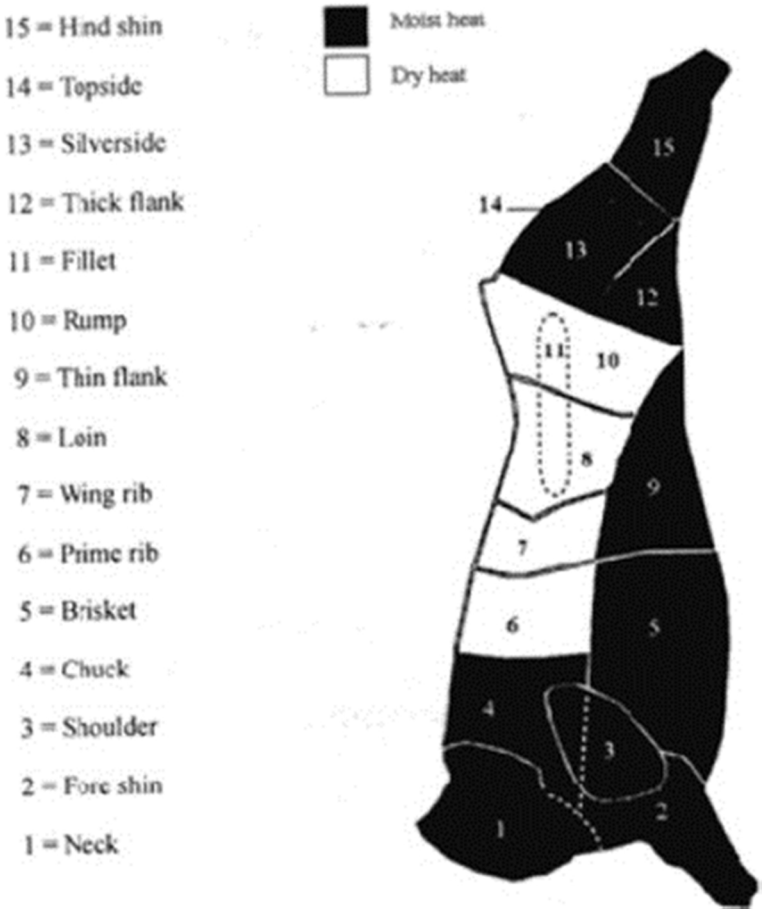

Fig. 1. The 15 retail cuts of South African beef.

A composite sample of each group of three similar cuts from the carcasses was used in the analysis. The 15 cuts from three age groups over six fatness levels resulted in 270 samples for raw analysis, and 270 samples for cooked analysis. The use of composite samples for analysis rather than individual samples was justified due to budget constraints and it is an accepted approach in food composition studies (Greenfield & Southgate, 2003).

The subcutaneous fat plus meat obtained from each of the identical cuts of the three left sides of each age and fatness group was cubed, thoroughly mixed and then minced first through a 5mm and then through a 2mm mesh plate. Each composite sample was then divided into the amounts required for the various analyses. The samples were stored at -40°C after coding and packaging and distributed to the laboratories responsible for the determinations of the raw meat at regular intervals. All the cuts from all the age groups were analysed on a double blind basis over a period of three years.

2.2. Proximate analyses

The proximate analyses of the cuts were carried out to determine the percentages of total moisture, fat (ethanol extracted), nitrogen (N x 6.25 = protein) and ash. The protein methodology was based on the Dumas Combustion method (Einarsson, Josefsson, & Lagerkvist, 1983). The samples were combusted at ± 1100 °C to 1350 °C and 10cm^3 of the sample gas was analyzed. A thermal conductivity cell detected the difference in thermal conductivity caused by the presence of nitrogen. The conversion factor of 6.25 was used in the calculation of the protein content (Jones *et al*., 1942).

2.3. Amino acid profile

Amino acid determination was carried out by high-performance liquid chromatography (HPLC), following the method of Einarsson, Josefsson and Lagerkvist (1983). Amino acid determination was performed during three separate hydrolyses. The amount of amino acid was expressed on a wet mass basis following each analysis.

During the first analysis, 17 amino acids comprising arginine, hydroxyproline, serine, aspartic acid, glutamic acid, threonine, glycine, alanine, tyrosine, proline, methionine, valine, phenylalanine, isoleucine, leucine, histidine and lysine were determined. An amount of ground, freeze-dried meat was weighed accurately and hydrolysed with 6N hydrochloric acid. Internal standard (α-amino- β-guanidinopropionic acid) was added to the hydrolysate, after which the hydrolysate was filtered. An aliquot of the hydrolysate was dried under nitrogen-flow. The hydrolysate was derivatized with FMOC reagent (9-fluorenylmethyl chloroformate), after which the amino acid content was determined by means of an HPLC (using an

AminoTag column) and, as the eluent, a tertiary gradient of pH, methanol and acetonitrile. Peak detection was carried out by means of a fluorescent detector.

During the second analysis Cystine was determined, following a procedure identical to the above except that, prior to hydrolysis, cystine was oxidised to cysteic acid with a peroxide formic acid solution. The addition and subsequent evaporation of hydrobromic acid reduced excess oxidising agents.

For tryptophan determination, an amount of ground, freeze dried meat was hydrolysed enzymatically using protease. After filtration through a 0,45µm filter, tryptophan was determined by means of HPLC, using an AminoTag column and, as the eluent, a blend of buffer methanol and acetonitrile. Peak detection was carried out by means of a fluorescence detector.

2.4. Statistical analysis

Data was statistically analysed using the GenStat for Windows (2003) statistical computer programme. Fat class and age were used as the main factor for each cut and tested at a significance level of 95% ($p \leq 0.05$). The significance of the variables measured (protein (N x 6.25), total amino acids, and individual amino acids) for each cut and fat code, and cut and age, was analysed using analysis of variance (ANOVA). Interactions were tested at the 5% level of significance ($p \leq 0.05$). If a main effect was significant, the Fishers' protected t-test Least Significant Difference (LSD) was applied, to determine the direction of the differences between mean values (Snedecor & Cochran, 1980). A correlation matrix was constructed to test the correlation between total amino acid content and protein as calculated from nitrogen multiplied by 6.25.

3. RESULTS

3.1. Effect of age and fat code on protein content (N x 6.25) of different cuts

In Table 2 the mean protein content (N x 6.25) for each cut in each age group and fat class is presented to illustrate the effect of cut, age and fatness on total protein content calculated from total nitrogen. Protein content differed in two of the 15 cuts with age (topside, shoulder), in five of the 15 cuts with fat

code (prime rib, silverside, topside, thick flank and chuck), as well as with the total carcass value. As expected, the protein content in the total carcass (calculated) decreased significantly with an increase in fat code ($p \leq 0.05$).

Table 2. Effect of age and fat code on protein content (N × 6.25) of different beef cuts.

Cut	Age				Fat code						
	***p*-value**	**Age A**	**Age AB**	**Age C**	***p*-value**	**FC1**	**FC2**	**FC3**	**FC4**	**FC5**	**FC6**
Prime rib	>0.05	19.23	20.43	19.33	0.017	20.36^{bc}	20.21^{bc}	21.91^{c}	18.85^{ab}	17.72^{a}	18.93^{ab}
Wing rib	>0.05	19.03	20.63	22.05	>0.05	20.76	19.33	22.65	21.14	18.84	20.70
Loin	>0.05	17.64	18.42	19.55	>0.05	18.77	20.08	19.01	18.86	17.47	17.00
Silverside	>0.05	18.33	17.86	17.62	<0.001	20.83^{d}	19.35^{cd}	18.26^{bc}	17.69^{bc}	16.48^{ab}	15.00^{a}
Rump	>0.05	17.74	18.76	16.41	>0.05	19.48	18.83	16.82	17.82	14.93	17.94
Topside	0.003	13.69^{a}	15.69^{b}	17.17^{b}	0.006	19.08^{b}	14.91^{a}	15.84^{a}	15.01^{a}	14.52^{a}	13.74^{a}
Fillet	>0.05	17.24	16.49	17.41	>0.05	20.17	16.93	15.70	15.13	20.27	14.09
Thick flank	>0.05	16.52	18.88	17.92	0.010	21.84^{c}	18.44^{b}	16.69^{ab}	17.58^{ab}	16.94^{ab}	15.13^{a}
Chuck	>0.05	18.75	19.44	18.16	0.024	19.66^{bc}	18.99^{bc}	20.40^{c}	17.51^{ab}	19.58^{bc}	16.55^{a}
Brisket	>0.05	16.90	18.66	18.63	>0.05	20.27	16.41	18.57	18.99	17.60	16.53
Neck	>0.05	19.28	19.46	19.05	>0.05	19.98	18.52	20.21	17.85	19.56	19.45
Shoulder	0.024	18.9^{a}	20.8^{b}	20.34^{b}	>0.05	21.03	20.14	20.99	19.11	20.22	18.61
Thin flank	>0.05	19.96	20.35	20.80	>0.05	20.91	20.86	20.41	19.98	20.17	19.90
Hind shin	>0.05	21.16	21.02	20.12	>0.05	20.21	19.22	21.41	20.71	22.75	20.29
Fore shin	>0.05	21.00	22.75	22.05	>0.05	22.71	21.50	21.99	21.92	22.25	21.24
Carcass*	>0.05	17.69	18.82	18.68	0.009	20.11^{c}	18.48^{b}	18.98^{bc}	17.91^{ab}	17.83^{ab}	17.07^{a}

^{abc} Means in the same row with different superscripts differ significantly ($p \leqslant 0.05$).

*Carcass values were calculated according to cut contribution.

Table 3. Effect of age and fat code on amino acid content of different beef cuts.

Cut	Age				Fat code						
	p-value	Age A	Age AB	Age C	*p*-value	FC1	FC2	FC3	FC4	FC5	FC6
Prime rib	0.047	16.64^{a}	18.39^{b}	17.26^{ab}	0.047	18.69^{b}	17.34^{ab}	18.80^{b}	15.99^{a}	16.53^{a}	17.24^{ab}
Wing rib	>0.05	17.27	19.79	19.22	>0.05	20.03	18.31	18.42	19.89	18.17	17.73
Loin	0.040	15.93^{a}	17.05^{ab}	17.99^{b}	>0.05	16.39	17.67	17.56	17.45	16.45	16.45
Silverside	>0.05	15.20	16.36	17.36	>0.05	18.07	16.22	17.14	14.47	17.69	14.24
Rump	>0.05	16.31	17.24	17.49	>0.05	18.05	17.17	17.23	17.70	16.47	15.46
Topside	0.003	13.44^{a}	16.79^{b}	15.77^{b}	0.008	18.61^{b}	14.84^{a}	15.22^{a}	15.41^{a}	14.75^{a}	13.18^{a}
Fillet	>0.05	14.37	16.23	16.91	>0.05	19.11	15.10	16.48	14.19	16.15	13.99
Thick flank	>0.05	15.42	17.70	16.11	0.008	20.72^{a}	16.94^{b}	16.51^{ab}	15.82^{ab}	15.13^{ab}	20.72^{c}
Chuck	>0.05	16.74	17.80	16.46	>0.05	17.34	16.24	17.88	16.86	18.37	15.30
Brisket	>0.05	16.47	17.06	16.28	>0.05	17.51	15.56	16.35	16.56	16.42	17.23
Neck	>0.05	16.35	17.38	16.99	>0.05	17.56	16.10	17.89	16.21	16.94	16.74
Shoulder	>0.05	16.50	18.39	18.04	>0.05	18.51	18.03	18.10	17.02	16.78	17.40
Thin flank	>0.05	17.51	18.44	18.48	>0.05	19.48	18.05	17.03	17.90	17.62	18.77
Hind shin	>0.05	17.54	17.58	16.90	>0.05	16.60	16.60	18.43	17.15	17.55	17.72
Fore shin	>0.05	17.91	19.74	19.90	>0.05	21.43	19.21	20.17	17.49	16.80	20.00
Carcass*	0.005	14.82^{a}	18.05^{b}	17.79^{b}	>0.05	18.33	15.33	16.43	16.42	16.58	18.23

^{abc} Means in the same row with different superscripts differ significantly ($p \leqslant 0.05$).

*Carcass values were calculated according to cut contribution.

3.2. Effect of age and fat code on amino acid content of different cuts

In Table 3 the total amino acid count for each cut in each age group and fat class is presented to illustrate differences in amino acid content between the cuts, age groups and fat codes. For the total carcass

(calculated), prime rib, loin and topside the total amino acid content differed significantly between the age groups ($p \leq 0.05$). Carcasses from younger animals (age A) had a significantly lower total amino acid count than the carcasses from older animals (age AB and age C). There was a significant difference ($p \leq 0.05$) observed in the total amino acid content between the six fat codes for three of the 15 cuts (prime rib, topside and thick flank).

Table 4. Calculated nitrogen to amino acid conversion factors for South African beef.*

	Age				Fat code						
Cut											
	Age A	**Age AB**	**Age C**	**Average**	**FC1**	**FC2**	**FC3**	**FC4**	**FC5**	**FC6**	**Average**
Prime rib	5.41	5.63	5.58	5.54	5.74	5.36	5.36	5.30	5.83	5.69	5.54
Wing rib	5.67	6.00	5.45	5.70	6.03	5.92	5.08	5.88	6.03	5.35	5.70
Loin	5.64	5.79	5.75	5.73	5.46	5.50	5.77	5.78	5.89	6.05	5.73
Silverside	5.18	5.73	6.16	5.68	5.42	5.24	5.87	5.11	6.71	5.93	5.68
Rump	5.75	5.74	6.66	6.03	5.79	5.70	6.40	6.21	6.89	5.39	6.03
Topside	6.14	6.69	5.74	6.18	6.10	6.22	6.01	6.42	6.35	6.00	6.18
Fillet	5.21	6.15	6.07	5.81	5.92	5.57	6.56	5.86	4.98	6.21	5.81
Thick flank	5.83	5.86	5.62	5.77	5.93	5.74	6.18	5.62	5.58	8.56	6.20
Chuck	5.58	5.72	5.66	5.66	5.51	5.34	5.48	6.02	5.86	5.78	5.66
Brisket	6.09	5.71	5.46	5.74	5.40	5.93	5.50	5.45	5.83	6.51	5.75
Neck	5.30	5.58	5.57	5.49	5.49	5.43	5.53	5.68	5.41	5.38	5.49
Shoulder	5.46	5.53	5.54	5.51	5.50	5.60	5.39	5.57	5.19	5.84	5.51
Thin flank	5.48	5.66	5.55	5.57	5.82	5.41	5.21	5.60	5.46	5.90	5.57
Hind shin	5.18	5.23	5.25	5.22	5.13	5.40	5.38	5.18	4.82	5.46	5.22
Fore shin	5.33	5.42	5.64	5.47	5.90	5.58	5.73	4.99	4.72	5.89	5.47
Carcass	5.24	5.99	5.95	5.74	5.70	5.18	5.41	5.73	5.81	6.67	5.74

[abc] Means in the same row with different superscripts differ significantly ($p \leqslant 0.05$).

*Conversion factors calculated as total amino acids (g) divided by total nitrogen (g).

3.3. Correlation between total amino acids and protein (N x 6.25)

For all 15 cuts the correlation coefficient of total amino acids to protein (N x 6.25) was only 0.635, indicating a poor correlation for predicting protein content (as determined by total amino acid count), based on nitrogen.

For all 15 cuts, from the three age groups and 6 fat codes included in the study, on average, the sum of amino acids per cut amounted to 91% of total determined protein (N x 6.25). Nitrogen-to-protein conversion factors were calculated for all cuts over age groups and fat classes by dividing total amino acid count (g) with total nitrogen (g). On average, a nitrogen-to-protein conversion factor of 5.74 was found for South African beef (Table 4). The topside cut had the conversion factor (6.18) closest to the Jones factor of 6.25, while the hind shin had the lowest correlation with a conversion factor of 5.22 compared to the Jones factor of 6.25.

4. DISCUSSION

4.1. Protein measurement in context of bioavailability and human nutrition

As the different sources of protein have diverse properties and physiological effects due to significant intrinsic chemical and structural differences, it is not possible to predict the nutritional effect of protein solely on the basis of proximate measures such as total nitrogen. The true nutritional value of dietary protein, irrespective of source, is only realized after ingestion (Robinson, 1987). Amino acids are the building blocks of proteins, and are required for numerous functions within the human body. During digestion and absorption, protein molecules are denatured and broken down to these amino acids by digestive enzymes. The free amino acids are absorbed through the gastro-intestinal wall into the bloodstream, where they are either used for energy, or reassembled into the different proteins required in the human body (Robinson, 1987; Whitney & Rolfes, 2010).

Within a human nutrition perspective, it should be mentioned that although measurement of total amino acids is considered more accurate than determining total nitrogen, neither of these procedures accurately determine the amount of available indispensable amino acids for metabolic functions (Lewis, & Bayley in

Ammerman & Baker, 1995). Bioavailability of protein refers to the proportion of the total amount of dietary amino acids which is absorbed and utilized in the human body (Penchartz, Elango, & Ball, 2011). The bioavailability of amino acids, as with most other nutrients, is complex and dependent on various factors. As the digestion of many dietary proteins is incomplete, and because there is inconstant entry of endogenous protein into the intestinal lumen, assessment of bioavailability of protein is rather difficult (Fuller & Tomé, 2005). In March 2011, the 1st International Symposium on Dietary Protein for Human Health was held in Auckland, New Zealand, followed by an FAO Protein Consultation during which the topic of protein bioavailability was re-highlighted. Measurements to determine protein digestibility and bioavailability are currently widely scrutinized, and although no international consensus has been reached, it is long known that all of these methods at some point require the amino acid content in foods as part of calculations (Eliot *et al.,* 1943).

4.2. Protein content determined by total nitrogen vs. total amino acid count

The results from the two analytical methods during this study indicated that on average, protein content (N X 6.25) overestimated total amino acid (TAA) content of South African beef. Although the Dumas method which was used to calculate total nitrogen, has been largely been replaced globally by the Kejldahl method, the nitrogen values obtained are still recognized. Amino acid count (g) amounted on average to 91% of total protein based on grams of nitrogen multiplied by 6.25. This correlation is similar to what has been found by many authors working on red meat, including Sales and Hayes (1996) on ostrich meat (91%), and Elgasim and Alkanhal (1992) on, beef (87%), lamb (91%), chicken (89%), fish (91%) and camel meat (87%). The best correlation between the total amino acid profile and total protein based on nitrogen (N X 6.25) was observed in the topside cut (99%). The worst correlation was observed in the hind shin (84%).

All amino acids contain an amine group, a carboxylic acid group and a side-chain that varies between different amino acids. The amine group nitrogen contains one nitrogen molecule. The side chains of most amino-acids are only made up of carbon, oxygen and hydrogen, although some amino acids (including lysine, tryptophan, histidine and arginine) contain additional nitrogen molecules. If large amounts of these

high nitrogen-containing amino acids are found in protein foods, the amount of nitrogen analyzed would be greater than in proteins where lower amounts of the high nitrogen-containing amino acids are found. In cuts, such as the hind shin, significant amounts of collagen and elastin are found, compared to “clean” muscle cuts such as the topside (Schönfeldt & Welgemoed, 1996).

Collagen represents 30% of total protein in animals, and glycine and proline account for about 50% of the amino acids in collagen. Other amino acids contributing significantly to collagen in beef (in varying amounts) include alanine, arginine, glutamic acid, aspartic acid, leucine, lysine and serine (Bolboaca & Jantschi, 2007). It could be that more high nitrogen-containing amino acids are found in the hind shin, increasing total protein as determined by nitrogen content to inaccurate amounts, or the high concentrations of collagen and elastin could have restricted homogenous sampling and complicated hydrolysis during amino acid determination. Furthermore, hemoglobin and myoglobin, which are found in blood, contain noteworthy amounts of nitrogen. These free nitrogen molecules could also increase the total nitrogen content in the sample, inaccurately overestimating total protein. Sosulski and Imafidon (1990) found that nitrogen-to-protein conversion factors for egg, meat, fish and cereal products varied between 5.61 and 5.93, and suggested that these lower values compared to the Jones factor, could be due to incomplete recovery of amino acids, amino acid losses during hydrolysis or the presence of nucleic acid nitrogen and non-amino-acid nitrogen molecules. Similarly, the current study calculated the nitrogen-to-protein conversion factor for South African beef to be on average 5.74 (Table 5), based on total amino acid content (g) divided by total nitrogen (g).

5. CONCLUSIONS AND RECOMMENDATIONS

Protein content of South African beef, as determined by nitrogen and multiplied by the conversion factor of 6.25, overestimated total amino acid content by approximately 9% on average. Although it seems as if the conversion factor of 6.25 for beef and other red meat might overestimate actual protein content, methodological constraints to determine amino acids, including lack of full recovery during hydrolysis and amino acid losses, need to be taken into consideration. Yet, in terms on human nutrition and the current

global agenda of protein bioavailability and digestibility, increased data on the amino acid profile of protein-rich foods is essential.

6. REFERENCES

Agricultural Product Standards Act. (1990). Statutes of the Republic Of South Africa-Agriculture. Act No.119 of 1990.

AOAC. (1995). *Official methods of analysis.* 16th ed. Washington. Assoc. Off. Anal. Chem..

Balboaca, S.D. & Jantschi, L. (2007). Amino acid sequence analysis on collagen. Bulletin USAMV-CN, 63-64, 311 – 316.

Einarsson, S., Josefsson, B. & Lagerkvist, S. (1983). Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase HPLC. *Journal of Chromatography*, 282, 609-618.

Elgasim E.A. & Alkanhal, M.A. (1992). Proximate composition, amino acids and inorganic mineral content of Arabian Camel meat: comparative study. *Food Chemistry*, 45 (1), 1-4.

Eliot, F.B., Beach, B.M. & Robinson, A. (1943). The amino acid composition of animal tissue protein. *The Journal of Biological Chemistry*, 148, 431-439.

Fuller, M.F. & Tomé, D. (2005). In vivo Determination of Amino Acid Bioavailability in Humans and Model Animals. *Journal of AOAC International*, 88 (3), 923-934.

GenStat. (2003). *GenStat Release 4.2 5th Edition*. VSN Internation Ltd., Oxford, UK.

Greenfield, H. & Southgate, D.A.T. (2003). Food composition data. Production, management and use. 2nd edtition. Food and Agriculture Organization of the United Nations. Rome.

Jones, D.B., Munsey, V.E. & Walker, L.E. (1942). Report of Committee on Protein Factors. *Journal of the Association of Official Analytical Chemists*, 25, 118 – 120.

Lewis, A.J & Bayley H.S in Ammerman, C.B & Baker, D.H. (1995). Bioavailability of nutrients for animals: amino acids, minerals, vitamins, Chapter 2: p35 – 37.

Sales, J. & Hayes, J.P. (1996). Proximate, amino acid and mineral composition of ostrich meat. *Food Chemistry*, 56(2), 167-170.

Snedecor, G.W. & Cochran, W.G. (1980). *Statistical methods* (Seventh Edition). Iowa State University Press.

Sosulski, F.W. & Imafidon, G.I. (1990). Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *Journal of Agricultural and Food Chemistry*, 38, 1351 – 1356.

Whitney, E.N. & Rolfes, S.R. (2010). *Understanding Nutrition 12th Edition*. Wadsworth Publishing

Schonfeldt, H.C. & Welgemoed, C. (1996). *Composition of South African beef.* South African Meat Board, Pretoria, South Africa.

Robinson, D.S. (1990). *Food - Biochemistry and Nutritional Value*. Longman Scientific & Technical. Copublished in the United States with John Wiley & Sons, Inc., New York.

Penchartz, P., Elango, R. & Ball, R.O. (2011). Abstract: Recent advances in protein and amino acid requirements. International Symposium on Dietary Protein for Human Health. Skycity Convention Centre, Auckland, New Zealand, 27 – 30 March 2011. Symposium Proceedings, 17 – 18.