Nitrogen vs. Amino-acid profile as indicator of protein content of South African beef

Nicolette G Hall & Hettie C Schönfeldt

Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa

nicolette.gibson@up.ac.za

INTRODUCTION AND OBJECTIVE

Food composition studies and tables use the proximate system of measuring protein as total nitrogen (determined by Kjedahl 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. Different factors, originally determined by Jones *et al.* (1942)[1], are currently used to calculate proximate protein amounts based on nitrogen content in different foods (Table 1).

Table 1: Factors for the conversion of nitrogen values to protein (per g N)[2]

Animal products		Plant products	
Foodstuff	**Factor**	**Foodstuff**	**Factor**
Meat and fish	6.25	Whole wheat	5.83
Gelatin	5.55	Rice and rice flour	5.95
Milk and milk products	6.38	Rye and rye flour	5.83
Casein	6.40	Oats	5.83
Human milk	6.37	Maize	6.25
Eggs (whole)	6.25	Beans	6.25
Eggs (albumin)	6.32	Soya	5.71
Eggs (Vitellin)	6.12	Almonds	5.18

It is considered more scientifically correct, although more expensive, to base estimates of protein content on amino acid data.[2] In order to ensure accuracy of this approach certain concerns such as free amino acids and soundness of analytical data need to be taken into account.

A study conducted on the 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. 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. A study was commissioned in which 15 cuts from carcasses of 3 age groups and 6 fatness classes were analyzed for total N as well as complete amino acid profiles.

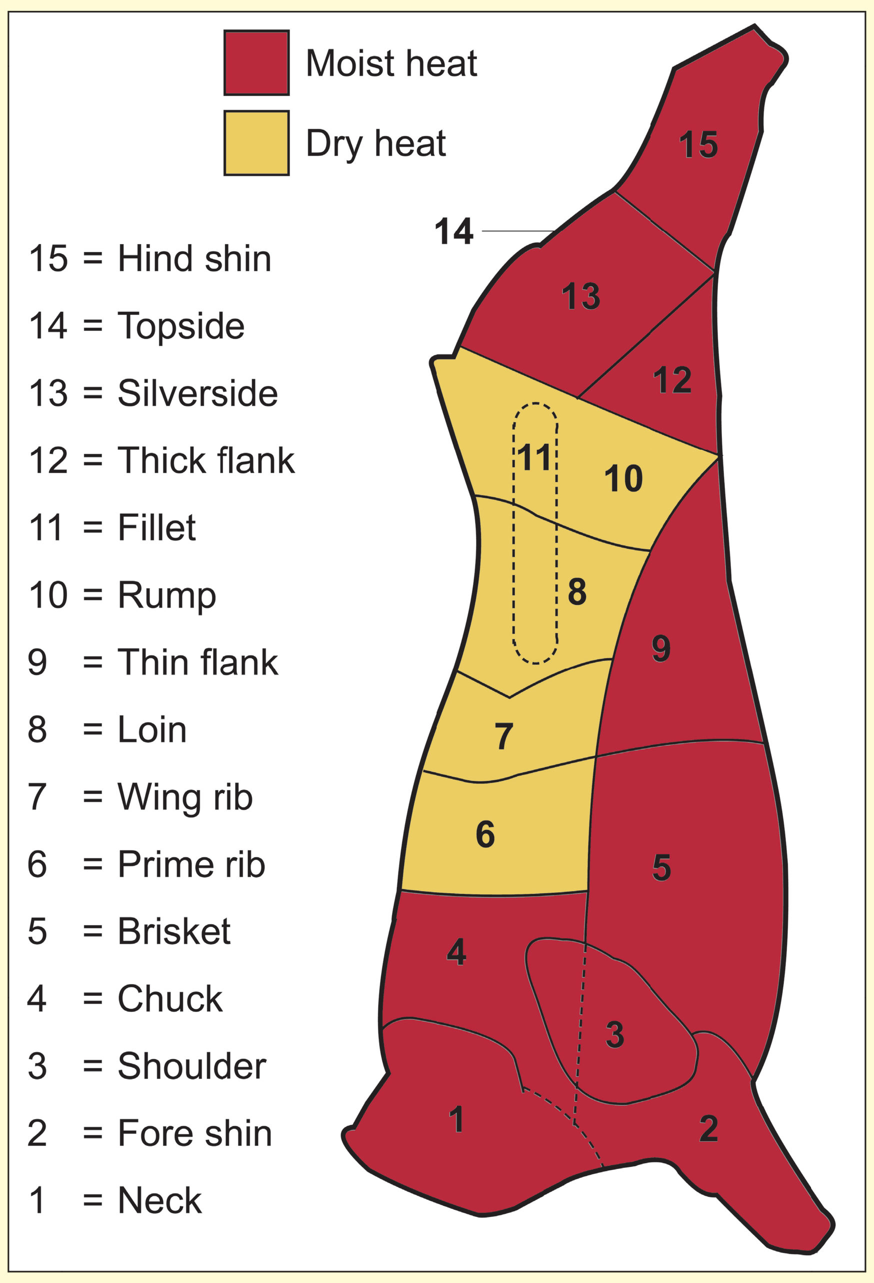

Figure 1: The 15 cuts of the beef carcass

METHODOLOGY

Sampling

Beef carcasses (190 - 240 kg) were selected to represent the commercial market and the South African classification system (based on age and carcass fatness regardless of genotype).[3] The three age groups were A) < 18 months, B) 18 to 24 months, and C) > 36 months. Carcasses representing the fatness spectrum (6 fat classes) within each age group were selected (Table 2).

Each of the sides of the carcasses was subdivided into 15 wholesale cuts (including the fillet) according to Figure 1. Each cut was then accurately weighed and dissected (at 10°C ambient temperature) into subcutaneous fat, meat (muscle and inter- and intra-muscular fat) and bone, in order to determine the physical composition of each cut and, by summation, the entire carcass. A composite sample of each group of three similar cuts from the carcasses was used in the analysis (3 age groups x 6 fatness levels x 15 cuts = 270 samples raw (left sides)). 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.[2]

Table 2: Classification system of beef carcasses in South Africa

Classification according to fatness of carcass		Classification according to age	
Fat Code	**Average fat percentage of carcass (g/100g)**	**Age group**	**Age of animal at slaughter (months)**
1	10.14	A	< 18
2	13.61		
3	14.86	B	18 to 24
4	19.00		
5	18.57	C	> 36
6	22.87		

The subcutaneous fat plus meat obtained from each of the identical cuts of the three right sides of each age and fatness group was cubed, thoroughly mixed and then minced first through a 5 mm and then through a 2 mm 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 were analysed on a double blind basis over a period of three years.

Proximate analyses

The proximate analyses of the cuts were carried out to determine the percentages of total moisture, fat (ether extracted), nitrogen (N x 6.25 = protein) and ash. The protein analysis was based on the Dumas Combustion method.[5] The samples were combusted at ±1100 °C – 1350 °C and 10 cm^3 of the sample gas was analyzed. The conversion factor of 6.25 was used in the calculation of the protein content.[1]

Amino acid profile

Amino acid determination was carried out by high-performance liquid chromatography (HPLC), following the method of Einarsson, Josefsson and Lagerkvist (1983)[5]. Amino acid determination was performed during three separate hydrolyses. The amount of amino acid was expressed on a wet mass basis following each analysis.

Hydrolysis 1: 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 6 N hydrochloric acid. Internal standard (α-amino and β-guandino propionic 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.

Hydrolysis 2: Cystine determination. The procedure followed was identical to the above except that, prior to hydrolysis, cysteine was oxidised to cystine by the addition of a peroxide-formic acid blend. The addition and subsequent evaporation of hydrobromic acid reduced excess oxidising agent.

Hydrolysis 3: For tryptophan determination, an amount of ground, freeze dried meat was hydrolysed enzymatically using protease. After filtration through a 0,45 µm 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 detection.

Statistical analysis

Data was statistically analysed using the GenStat for Windows (2003)[5] statistical computer programme. Fat class and age were used as the variable 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)[7]. A correlation matrix was constructed to test the correlation between total amino acid content and protein, calculated as nitrogen (N) multiplied by 6.25.

RESULTS

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

For the carcass, 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 B 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 3).

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 B	Age C	p-value	FC1	FC2	FC3	FC4	FC5	FC6
Prime rib	0.0470	16.64[a]	18.39[b]	17.26ab	0.0470	18.69[b]	17.34ab	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	0.0400	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	0.0030	13.44[a]	16.79[b]	15.77[b]	0.0080	18.61[b]	14.84a	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	0.0080	20.72[a]	16.94b	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*	0.0050	14.82[a]	18.05[b]	17.79[b]	> 0.05	18.33	15.33	16.43	16.42	16.58	18.23

*Carcass values were calculated according to cut contribution

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

Protein content differed in two of the 15 cuts with age (topside, shoulder), and in five of the 15 cuts with fat code (prime rib, silverside, topside, thick flank and chuck), along with the carcass value. The protein content in the carcass decreased significantly with an increase in fat code ($p \leq 0.05$) (Table 4).

Table 4: Effect of age and fat code on protein content (N X 6.25) of different beef cuts

Cut	Age				Fat code						
	P-value	Age A	Age B	Age C	p-value	FC1	FC2	FC3	FC4	FC5	FC6
Prime rib	> 0.05	19.23	20.43	19.33	0.0170	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	0.0030	13.69[a]	15.69[b]	17.17b	0.0060	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	0.0100	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	0.0240	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	0.0240	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	0.0090	20.11[c]	18.48[b]	18.98[bc]	17.91[ab]	17.83[ab]	17.07[a]

*Carcass values were calculated according to cut contribution

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

For all the 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 actual protein content, as determined by total amino acid count, based on the nitrogen factor (6.25).

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). In Table 5 the correlations (percentage) of total amino acids to protein (N x 6.25) are presented for each cut, age group and fat code.

Table 5: Relationship (%) of total amino acids to protein (N X 6.25)*

Cut	Age				Fat code						
	Age 1	Age 2	Age 3	Average	FC1	FC2	FC3	FC4	FC5	FC6	Average
Prime rib	86.53	90.01	89.29	88.61	91.80	85.80	85.81	84.83	93.28	91.07	88.76
Wing rib	90.75	95.93	87.17	91.28	96.48	94.72	81.32	94.09	96.44	85.65	91.45
Loin	90.31	92.56	92.02	91.63	87.32	88.00	92.37	92.52	94.16	96.76	91.86
Silverside	82.92	91.60	98.52	91.02	86.75	83.82	93.87	81.80	107.34	94.93	91.42
Rump	91.94	91.90	106.58	96.81	92.66	91.18	102.44	99.33	110.31	86.18	97.02
Topside	98.17	107.01	91.85	99.01	97.54	99.53	96.09	102.66	101.58	95.92	98.89
Fillet	83.35	98.42	97.13	92.97	94.74	89.19	104.97	93.79	79.67	99.29	93.61
Thick flank	93.34	93.75	89.90	92.33	94.87	91.87	98.92	89.99	89.32	136.95	100.32
Chuck	89.28	91.56	90.64	90.49	88.20	85.52	87.65	96.29	93.82	92.45	90.65
Brisket	97.46	91.43	87.39	92.09	86.38	94.82	88.05	87.20	93.30	104.23	92.33
Neck	84.80	89.31	89.19	87.77	87.89	86.93	88.52	90.81	86.61	86.07	87.80
Shoulder	87.30	88.41	88.69	88.14	88.02	89.52	86.23	89.06	82.99	93.50	88.22
Thin flank	87.73	90.61	88.85	89.06	93.16	86.53	83.44	89.59	87.36	94.32	89.07
Hind shin	82.89	83.63	84.00	83.51	82.14	86.37	86.08	82.81	77.14	87.33	83.65
Fore shin	85.29	86.77	90.25	87.43	94.36	89.35	91.72	79.79	75.51	94.16	87.48
Carcass	83.78	95.91	95.24	91.64	91.15	82.95	86.56	91.68	92.99	106.80	92.02
Average	88.49	92.43	91.67	**90.86**	90.84	89.13	90.88	90.39	91.36	96.60	**91.53**

*Relationship was determined as total amino acid ÷ protein (N X 6.25) x 100

CONCLUSIONS AND RECOMMENDATIONS

Protein content of South African beef, as determined by nitrogen and multiplied by the conversion factor of 6.25, overestimates total amino acid content by approximately 9 percent on average. To ensure accurate protein content values, it could thus be recommended to determine the full amino acid profile of beef, rather than to determine nitrogen content and multiplying this by the Jones factor of 6.25.

REFERENCES

1. Jones DB, Munsey VE & Walker LE. 1942. Report of Committee on Protein Factors. J. Assoc. Off. Agric. Chem, 25: 118 – 120.
2. Greenfield H & Southgate DAT. 2003. Food composition data. Production, management and use. 2nd edtition. Food and Agriculture Organization of the United Nations. Rome.
3. SAMIC (SOUTH AFRICAN MEAT INDUSTRY COMPANY). s.a. Introduction:1-31 and Classification of Red Meat – A key to more effective marketing. Pretoria. Available online: www.samic.co.za. Accessed March 2008
4. AOAC (1995). Official methods of analysis. 16th ed. Washington. Assoc. Off. Anal. Chem..
5. Einarsson, S., Josefsson, B. & Lagerkvist, S. (1983). Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase HPLC. J. Chrom., 282, 609-618.
6. GenStat (2003) GenStat Release 4.2 5th Edition. VSN Internation Ltd., Oxford, UK.
7. SNEDECOR, G.W. & COCHRAN, W.G. 1980. Statistical methods (Seventh Edition.). Iowa State University Press. ISBN 0-8138-1560-6.