Nutrient content of sorghum beer strainings

I.V. van Heerden

National Food Research Institute, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria, 0001 Republic of South Africa

Received 4 April 1986

A study was carried out to determine what contribution the consumption of sorghum beer strainings (SBS) could make towards animal nutrition. Sorghum beer strainings were analysed for starch, protein, fat, crude fibre, ash, minerals and phytate content on a dry mass basis. The amino acid composition of the protein was also determined. The carbohydrate content, the gross energy, digestible energy and apparent metabolizable energy were calculated using appropriate factors. In comparison with maize and sorghum, the strainings have a high protein (average 252 g/kg), crude fibre (50 g/kg) and gross energy content (19,52 MJ/kg). Strainings have a higher manganese and iron content, a similar copper, zinc, calcium, amino acid and fat content, and a lower carbohydrate, digestible energy, ash, magnesium, potassium and phosphorus content than maize and sorghum. The phytate content of sorghum beer strainings is lower than in sorghum so that it is possible that the bioavailability of the minerals of strainings is enhanced. Feeding trials should be carried out before recommendations can be made regarding incorporation of SBS in poultry, pig or ruminant diets.

'n Ondersoek is gedoen om vas te stel watter bydrae sorghumbierdoppe tot die voedingsbehoeftes van diere kan maak. Sorghumbierdoppe is ontleed om die stysel-, proteïen-, vet-, ruvesel-, mineraal-, as- en fitaatinhoud (op 'n droë basis) te bepaal. Die aminosuursamestelling van die proteïen is ook bepaal. Die koolhidraatinhoud, die bruto energie, die verteerbare energie en die skynbaar metaboliseerbare energie is met behulp van gepaste faktore bereken. In vergelyking met mielies en sorghum, het bierdoppe 'n hoë proteïen- (gemiddeld 252 g/kg), ruvesel- (50 g/kg) en bruto energie-inhoud (19,52 MJ/kg). Bierdoppe het 'n hoër mangaan- en ysterinhoud, dieselfde koper-, sink-, kalsium-, aminosuur- en vetinhoud, en 'n laer koolhidraat-, verteerbare energie-, as-, magnesium-, kalium- en fosforinhoud as mielies en sorghum. Die fitaatinhoud van bierdoppe is laer as in sorghum en die biobeskikbaarheid van die minerale in bierdoppe is moontlik hoër. Voedingseksperimente moet uitgevoer word, voordat aanbevelings betreffende die insluiting van sorghumbierdoppe in pluimvee, vark- of herkouerrantsoene, gemaak kan word.

Keywords: Nutrient composition, sorghum beer strainings, gross energy, digestible energy, crude fibre, protein, amino acids

The brewing of sorghum beer is unique to southern Africa. Sorghum beer is made from sorghum malt and a starchy adjunct such as maize grits or sorghum. After mashing, the coarser particles (>250 μ) are removed in a decanting centrifuge using a differential straining process. These strainings can be compared to spent grains which are a byproduct of barley beer brewing.

In South Africa, sorghum beer strainings are primarily used as animal feeds. As a result of the drought and the expected population expansion, all locally available feedstuffs should be utilized as completely as possible. An understanding of the nutrient composition of sorghum beer strainings would assist farmers and balanced-feed manufacturers in compiling balanced diets for livestock. Very little research has been carried out to determine the nutritive content of the strainings.

Three studies have previously reported on the nutrient composition of sorghum beer strainings. Bechman & Luetchford (van Heerden, 1981) studied the protein content and its availability in strainings subjected to various drying techniques. The protein availability was determined with *Tetrahymena pyriformis*. Protein contents varied between 18,9 and 25,2% on a dry mass basis. The protein availability depended on the technique used to dry the strainings. The results of proximate and vitamin B analyses of sorghum beer strainings were reported by van Heerden (1981). Strainings were found to be a good source of carbohydrate, protein, crude fibre, energy and nicotinic acid. Taylor & Glennie (Novellie & De Schaepdrijver, 1986) reported an average protein content of 26,9% and an average starch content of 44,1% on a dry mass basis. These authors found that the strainings protein contained 1,5% of the essential amino acid lysine; other essential amino acids were not reported. In the present study, sorghum beer strainings were analysed for all amino acids except tryptophan.

The studies cited above did not report on the gross energy, digestible energy, apparent metabolizable energy, mineral and phytate content of the strainings. The importance of minerals in animal nutrition has been recognized for many years. The bioavailability of minerals in cereals can be decreased by the presence of phytate (Maga, 1982). Phytate occurs naturally in most grains and can bind certain minerals, e.g. magnesium, calcium, iron, zinc and copper, thus decreasing their bioavailability.

Experimental procedure

Eighteen samples of sorghum beer strainings were obtained from ten breweries situated in Somerset West, Port Elizabeth, Durban, Johannesburg, Pretoria, Alberton, Randfontein, Witbank, Nelspruit and Pietersburg. Eleven samples were chosen at random for complete nutrient assay. Past experience (van Heerden, 1985) had shown that samples of sorghum beer strainings exhibit a very wide variation in mineral content. Consequently, the mineral and phytate assays were performed on all 18 samples.

Samples of wet strainings were dried in stainless steel containers in a forced-draught oven (Labotec Therm-O-Mat) at 50°C for 16 hours. The dry samples were ground in a hammermill (Janke and Kunkel, Staufen, West Germany). Total solids of the samples were determined before and after drying with a vacuum oven (Heraeus, Hanau, West Germany) at 70°C and 220 mm Hg over 16 hours (AOAC, 1975).

Protein (N × 6,25), was determined by the method of Thomas, Sheard & Moyer (1967). The amino acid composition of hydrolyzed strainings was determined using a 4150 LKB amino acid analyser. The samples were hydrolyzed under vacuum with 6 M HCl at 110°C for 22 hours (Davies & Thomas, 1973). Starch was determined according to a method described by Faure (Novellie & De Schaepdrijver, 1986). The starch of the strainings samples was hydrolyzed to glucose in the presence of α -amylase and amyloglucosidase (Novo Industries, Copenhagen, Denmark). Glucose was oxidized to gluconic acid using glucose oxidase (Boehringer, Mannheim, West Germany). The reaction was coupled to dye reduction with peroxidase (Miles Seravac, Cape Town, SA).

Fat was determined by soxhlet extraction with hexane for 6 hours. A Fibertec hot extractor was used to digest the strainings samples with 0,1275M sulphuric acid, followed by 0,3125M sodium hydroxide to obtain the crude fibre residue (AOAC, 1975).

After the samples had been dry-ashed at 520°C minerals, except for phosphorus, were analyzed by atomic absorption using a Perkin Elmer Atomic Absorption Spectrophotometer (AOAC, 1975). The phosphorus content of the dry-ashed samples was determined according to the sodium molybdate-

hydrazine sulphate technique (Boltz & Mellon, 1947). Phytate was extracted with 3% aqueous trichloroacetic acid, and separated by HPLC using an in-line post column colorimetric reaction for detection (Cilliers & van Niekerk, 1986). To estimate the phosphorus component of the phytate in the samples, the phytate content in mg/kg was multiplied by a conversion factor of 0,282 (Number of P molecules in phytate × MW of P ÷ MW of phytate = $(6 \times 31/660) = 0.282$).

The total carbohydrate content of the moisture-free strainings samples was calculated by difference, i.e. the sum of the protein, fat, ash and crude fibre contents was subtracted from 100 for each sample (Wilson, Fisher & Fuqua, 1975). The gross energy content of the samples was calculated as follows: Carbohydrate and crude fibre $\times 17,4$; protein $\times 23,6$; fat $\times 39,3$ (Whittemore & Elsley, 1976). The energy content of strainings was calculated in terms of digestible energy (DE) for pigs. A formula suggested by Whittemore (1985), namely, DE = 17 + 0,011(fat content in g/kg) – 0,041(crude fibre content in g/kg) was used. The apparent metabolizable energy (AME) of the strainings was calculated using a formula cited by Fisher (1982), namely,

AME = 31,36(fat content in g/g) + 12,75 (crude protein in g/g) + [1 - (fat + crude protein + crude fibre + ashcontents in g/g)] [15,30 - 38,1 (crude fibre content in g/g)].

Results

Table 1 shows the moisture content prior to drying, as well as the carbohydrate, starch, fat, protein, crude fibre, ash and energy contents on a dry matter (DM) basis of eleven samples of sorghum beer strainings. The amino acid composition of the same strainings samples is shown in Table 2. The mineral, phytate, total

Table 1 Nutrient content of eleven samples of sorghum beer strainings

		Nutrient content (per kg DM basis)										
Strainings	Moisture content before drying	Starch	Fat	Protein	Crude fibre	Ash	Gross energy	Digestible energy (DE)	Apparent metabo- lizable energy (AME)			
sample no.	%	g	g	g	g	g	g	MJ	MJ	MJ		
1	60,3	684	441	23	227	59	7	19,21	14,83	12,54		
2	60,8	655	447	31	258	50	6	19,60	15,29	13,04		
3	60,5	624	441	30	278	60	8	19,67	14,87	12,61		
4	64,9	644	490	37	261	48	10	19,68	15,44	13,16		
5	74,7	619	406	31	278	63	9	19,67	14,76	12,50		
6	57,9	657	455	28	266	44	5	19,60	15,50	13,22		
7	60,9	678	469	33	233	50	6	19,49	15,31	13,09		
8	56,9	707	490	26	215	47	5	19,24	15,36	13,11		
9	60,8	646	469	32	263	48	11	19,57	15,38	13,06		
10	61,5	670	478	31	248	41	10	19,47	15,66	13,34		
11	62,3	668	431	32	249	42	9	19,51	15,63	13,33		
Mean	62,0	659	456	30	252	50	7,8	19,52	15,28	12,99		
SD	4,7	26	26	3,7	20	7,4	2,1	0,16	0,32	0,31		

phosphorus and phytate phosphorus contents of all 18 samples are presented in Table 3.

Discussion

Feed manufacturers and farmers usually calculate animal diets on an air-dry basis, i.e. the diets contain 10–14% moisture (Kemm, 1985). Sorghum beer strainings, are however, sold while still wet and can contain up to 75% moisture (Table 1). To utilize the results of this study it is necessary to determine the average moisture content of the strainings (e.g. 75%) produced by a specific brewery and to multiply the mean values in the tables by the relevant factor.

The results of this study show that sorghum beer strainings have a mean carbohydrate content of 659 g/kg (Table 1). The carbohydrate content consists of 69% starch, i.e. 456 g/kg which was slightly higher than that reported by Taylor & Glennie (Novellie & De Schaepdrijver, 1986). The carbohydrate content of strainings is approximately 20% lower than that of maize or sorghum (Souci, Fachmann & Kraut, 1981; Wehmeyer, 1969). The strainings had a mean fat content of 30 g/kg (Table 1) which is similar to that of sorghum and lower than in maize (Wehmeyer, 1969; Souci, *et al.*, 1981).

The mean protein content of 252 g/kg of strainings is approximately double the value found in the original grains used to brew sorghum beer, i.e. 105 g/kg for maize and 123 g/kg for sorghum (Souci, *et al.*, 1981; Wehmeyer, 1969). Comparison of the mean protein content of sorghum beer (van Heerden, 1981) and that of strainings shows that the differential strainings process used during beer production increases the protein content of the strainings. It would seem that strainings are a good source of protein for inclusion in animal feeds. However, when the amino acid composition of the strainings is examined (Table 2), it appears that the lysine content of the protein in the strainings probably limits the value of this protein. The mean lysine content of strainings protein is similar to that of sorghum (1,9 vs. 2,2 g per 100 g protein) (Srinivasan, Axtell & Jambunathan, 1972). Feeds using maize, sorghum and sorghum beer strainings would most probably require lysine supplementation to ensure optimum growth.

Since both maize and sorghum are limiting in tryptophan, the limiting effect of this amino acid in strainings is very likely, unless tryptophan is produced during beer souring by Lactobacillus or during beer fermentation by Saccharomyces cerevisiae. Tryptophan was unfortunately not determined in this study due to difficulties experienced with the assay and in future work, attempts should be made to include this amino acid in the analyses. The bioavailability of proteins in strainings to animals may also be affected by the tannin content and drying methods employed. Although formaldehyde treatment of sorghum grain to reduce the tannin content substantially increased the apparent protein digestibility, N retention by pigs was adversely affected (Kemm, Ras & Daiber, 1984). It is therefore possible that when sorghum beer strainings which are derived from formaldehyde-treated sorghum grain are used in animal feeds, a similar decrease in N retention may occur. The decreased protein digestibility caused by drying of strainings reported by Bechman & Luetchford (van Heerden, 1981), may also affect the protein quality adversely.

 Table 2 Amino acid composition of eleven samples of sorghum beer strainings (results expressed in g/100 g protein)

	Amino acid composition of sorghum beer strainings												
Amino acid	1	2	3	4	5	6	7	8	9	10	11	Mean	SD
Aspartic acid	6,4	6,5	6,2	6,2	6,6	6,6	6,2	6,2	6,2	6,3	6,3	6,3	0,16
Threonine	3,3	3,5	3,4	3,3	3,4	3,4	3,4	3,4	3,5	3,3	3,4	3,4	0,07
Serine	4,3	4,3	4,3	4,2	4,2	4,2	4,3	4,2	4,4	4,2	4,3	4,3	0,07
Glutamic acid	19,9	19,3	19,7	19,8	19,5	19,3	19,8	20,2	19,8	20,2	19,3	19,7	0,33
Proline	10,0	9,6	10,1	9,9	9,9	9,7	9,5	9,8	9,6	9,6	9,5	9,7	0,21
Glycine	3,0	3,1	3,1	3,1	3,0	2,9	3,1	3,0	3,0	3,0	3,0	3,0	0,07
Alanine	8,4	8,8	8,6	8,8	8,7	8,6	8,3	8,5	8,6	8,6	8,6	8,6	0,15
Cystine	1,7	1,6	1,8	1,7	1,7	1,9	1,8	1,7	1,8	1,8	1,7	1,7	0,08
Valine	5,0	5,0	5,0	5,1	5,0	4,9	5,0	5,1	5,1	5,2	5,0	5,0	0,08
Methionine	1,9	2,1	1,9	1,9	1,9	2,1	2,0	2,1	2,0	1,9	2,2	2,0	0,11
Isoleucine	4,4	4,3	4,4	4,5	4,5	4,4	4,3	4,1	4,3	4,2	4,5	4,4	0,13
Leucine	12,6	13,0	12,1	12,4	12,1	12,3	12,5	12,6	12,4	12,3	12,7	12,5	0,27
Tyrosine	3,1	3,3	3,3	3,2	3,4	3,5	3,4	3,3	3,3	3,3	3,4	3,3	0,11
Phenylalanine	5,5	5,9	5,7	5,8	5,8	5,8	5,8	5,6	5,7	5,7	5,8	5,7	0,11
Histidine	3,0	2,8	2,9	2,8	2,9	2,9	3,1	2,9	2,9	2,9	3,0	2,9	0,09
Lysine	2,0	1,8	2,0	1,9	1,9	1,7	1,9	1,8	1,8	1,8	2,0	1,9	0,10
Ammonia	2,1	2,0	2,1	2,1	2,0	2,1	2,1	2,2	2,1	2,1	1,9	2,1	0,08
Arginine	3,4	3,1	3,4	3,3	3,4	3,7	3,5	3,5	3,5	3,5	3,4	3,4	0,15

The dry strainings had a mean crude fibre content of 50 g/kg (Table 1), which could be useful in ruminant feeds. In comparison to maize and sorghum, strainings contain nearly double the amount of crude fibre, i.e. 25 and 27 g/kg for maize and sorghum respectively (Souci,*et al.*, 1981; Wehmeyer, 1969). The relatively high crude fibre content may cause problems in poultry feeding (Heuser, 1955). The carbohydrate, fat, protein and crude fibre results of the present study differ considerably from the results reported by van Heerden in 1981. These differences are probably due to variations in technique and components, which are known to occur at sorghum beer breweries on a day-to-day and seasonal basis.

The dry strainings had a mean gross energy content of 19,52 MJ/kg, a mean, calculated DE of 15,28 MJ/kg, and a mean AME of 12,99 MJ/kg. The gross energy content of strainings is slightly higher, while the calculated DE is slightly lower than the mean values calculated for maize and sorghum. The mean AME of the strainings should provide adequate energy for all types of poultry (NRC, 1984) provided the crude fibre content does not interfere with digestibility. Although the tannin content of the strainings was not determined in this study, a high tannin content could have an adverse effect on the DE. Although formaldehyde treatment of sorghum prior to brewing may decrease the nitrogen retention, it would probably improve the DE of strainings substantially (Kemm, *et al.*, 1984).

An examination of the mineral and phytate content of the strainings samples (Table 3), reveals that there is a wide variation in the results. This is probably due to the variation in brewing techniques mentioned above.

The ash content of strainings is lower than in sorghum malt, maize and sorghum grain, which probably accounts for the finding that some of the mineral values, such as those for magnesium, potassium and phosphorus, are considerably lower than in the other grains (van Heerden, 1985; Souci, *et al.*, 1981; Wehmeyer, 1969). Strainings are rich in manganese and have a high iron content. The origin of this iron is at present unknown. Iron contamination during brewing should not occur as the commercial brewing process makes use of stainless steel vessels. Such iron contamination may occur after straining.

There is a wide variation between the samples in the amount of phosphorus bound to phytate (Table 3). It was found that, on average, 42% of the total phosphorus occurred in the phytate form. In sorghum grain, 73 to 87% of the phosphorus is bound to phytate (Doherty, Faubion & Rooney, 1982). The lower average phytate of strainings is probably due to yeast activity during fermentation (Reddy, Sathe & Solunkhe, 1982). Hence, it is possible that the minerals in strainings are more bioavailable than in sorghum grain. Strainings should, however, be supplemented with minerals when used for animal feeds.

The above discussion of the nutritive content of dry sorghum beer strainings, is based on results obtained with chemical analyses. It is abundantly clear, however, that the final evaluation of sorghum beer strainings can

Table 3 Mineral and phytate content of sorghum beer strainings (results expressed in mg/kg of moisture-free sample)

Strainings	Minerals (mg.kg ⁻¹)										Phytate	Percentage
	Cu	Fe	Zn	Mn	Ca	Mg	К	Na	Р	(mg.kg ⁻¹)	(mg.kg ⁻¹)	of total P
1	10,0	141,8	27,8	8,6	163	330	517	75	1569	800	226	14
2	9,6	114,0	19,8	7,6	256	622	1095	129	2923	10400	2933	100
3	4,9	75,9	16,8	3,8	191	274	572	75	1531	900	254	17
4	4,4	64,1	20,7	4,1	134	312	537	89	1767	4800	1354 ·	77
5	7,8	9,5	19,1	3,9	71	227	463	86	1467	2200	620	42
6	3,5	68,1	16,5	5,6	111	205	813	49	1170	500	141	12
7	3,9	76,2	20,5	7,8	436	289	932	76	1722	3500	987	57
8	3,8	87,3	25,4	8,4	246	235	738	57	1542	1000	282	18
9	4,2	149,0	18,3	6,7	378	188	551	53	1323	1100	310	23
10	3,1	97,0	22,8	7,8	220	309	971	41	1355	1600	451	33
11	3,1	245,2	5,5	7,2	412	213	794	44	1256	1800	508	40
12	7,5	495,2	34,0	16,5	326	350	1170	116	1831	1000	282	15
13	5,0	73,0	14,2	5,3	314	200	725	30	1363	2500	705	52
14	4,9	78,9	15,7	6,8	241	303	958	18	1456	2100	592	41
15	4,4	82,0	13,2	7,2	222	261	833	18	1315	2100	592	45
16	3,1	144,4	18,6	12,0	136	295	544	80	1740	3500	987	57
17	5,6	161,2	17,3	12,5	135	296	479	51	2032	4000	1128	56
18	5,1	147,0	15,7	15,3	138	282	441	25	1677	3700	1043	62
Mean	5,2	128,3	19,0	8,2	229,4	288,4	729,6	61,8	1613,2	2640	744,2	42,3
SD	2,1	105,3	6,1	3,7	107,4	95,9	229,0	31,6	397,1	2310,0	651,3	24,0

only be done by conducting feeding trials with different types and classes of animals.

Conclusions

The results of this study show that the nutritive composition of dry sorghum beer strainings compares favourably with well-known feed grains such as maize and sorghum. Additional research with feeding trials is recommended. Hopefully, such trials will result in the increased use of sorghum beer strainings in animal feeds, which would benefit the sorghum beer industry, as well as the animal industries.

Acknowledgement

Mrs L. Schüssler is thanked for carrying out the protein and amino acid analyses.

References

- AOAC, 1975. In: Official Methods of Analysis of the Association of Official Analytical Chemists, 12th ed., Ed. Horwitz, W., Washington, D.C.
- BOLTZ, D.F. & MELLON, M.G., 1947. Determination of phosphorus, germanium, silicon and arsenic by the heteropoly blue method. *Anal. Chem.*, 19, 873.
- CILLIERS, J.J.L. & VAN NIEKERK, P.J., 1986. L.C. determination of phytic acid in foods by post-column colorimetric detection, J. Agric. Food Chem., 34, 680.
- DAVIES, M.G. & THOMAS, A.J., 1973. An investigation of hydrolytic techniques for the amino acid analysis of foodstuffs. J. Sci. Fd. Agric., 24, 1525.
- DOHERTY, C., FAUBION, J.M. & ROONEY, L.W., 1982. Semi-automated determination of phytate in sorghum and sorghum products. *Cereal Chem.*, 59, 373.
- FISHER, C., 1982. Energy evaluation of poultry rations. In: Recent Advances in Animal Nutrition, Ed. Haresign, W., Butterworth, London. pp. 113–134.
- HEUSER, G.F., 1955. Feeding Poultry. 2nd ed., John Wiley & Sons, New York, p.360.
- KEMM, E.H., 1985. Die energiebehoeftes van die groeiende vark. Meat Board Focus, 4, 22.
- KEMM, E.H., RAS, M.N. & DAIBER, K.H., 1984. Nutrient digestibility and performance of pigs fed sorghum varying in polyphenol concentration and maize as grain sources. S. Afr. J. Anim. Sci., 14, 1.
- MAGA, J.A., 1982. Phytate: its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. J. Agric. Fd., Chem., 30, 1.

- NRC (1984). Nutrient requirements of poultry. 8th ed., National Academy Press, Washington, D.C.
- NOVELLIE, L. & DE SCHAEPDRIJVER, P., 1986.
 Modern developments in traditional African beers. In: Progress in Industrial Microbiology, Ed., Adams, M.R., Vol. 23, pp.73–157, Elsevier Science Publishers, The Netherlands.
- REDDY, N.T., SATHE, S.K. & SOLUNKHE, D.K., 1982.
 Phytates in legumes and cereals. In: Advances in Food Research. Eds. Chichester, C.O., Mrak, E.M. & Stewart, G.F., Vol. 28, pp.1-75, Academic Press, New York.
- SOUCI, S.W., FACHMANN, W. & KRAUT, H., 1981. Food Composition and Nutrition Tables 1981/82. 2nd Ed., Eds.
 Deutsche Forschungsanstalt f
 ür Lebensmittelchemie, Wissenschaftliche Verlaggesellschaft, Stuttgart.
- SRINIVASAN, G., AXTELL, J.D. & JAMBUNATHAN,
 R., 1972. Amino acid composition of sorghum grain and relationships between amino acids and protein. In:
 Research progress report on inheritance and improvement of protein quality and content of Sorghum bicolor (L.)
 Moench. Report No. 9, Department of Agronomy, Purdue University, Lafayette, Indiana.
- THOMAS, R.L., SHEARD, R.W. & MOYER, J.R., 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium. Analysis of plant material using a single digestion. Agron. J., 59, 240.
- VAN HEERDEN, I.V., 1981. The Nutritive Content of Sorghum Beer. D.Sc. Thesis, University of Pretoria, Pretoria, S.A.
- VAN HEERDEN, I.V., 1985. Mineral, trace element and phytate composition of sorghum malt, beer and strainings. In: Proc. SAAFoST 85, Vol. 1., pp. 216–227. CSIR, Symposium Secretariat. Pretoria, S.A.
- WEHMEYER, A.S., 1969. Composition of kaffircorn (including hybrids). National Chemical Research Lab. . Report C. Chem 220, CSIR. Pretoria, S.A.
- WHITTEMORE, C.T., 1985. The application of the principles of nutrition to the feeding of breeding sows and the production of meat from growing pigs, S. Afr. J. Anim. Sci., 15, 97–101.
- WHITTEMORE, C.T., & ELSLEY, F.W.H., 1976. Practical Pig Nutrition. p. 57, Farming Press, Ipswich., Suffolk.
- WILSON, E.D., FISHER, K.H. & FUQUA, M.E., 1975. Principles of Nutrition. 3rd ed., Chap. 8, pp. 123–149. John Wiley & Sons, New York.