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The Choline Content of Feed Ingredients and Mixed Feeds Determined by an Enzymatic Assay

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Abstract: A recently developed procedure for the determination of choline in ingredients and feeds was utilised to establish the levels and the variability of choline in a series of ingredients and to compare the analysed and calculated choline values of mixed feeds. The concentrations of choline in samples of maize (7), canola meal (3), wheat middlings (1) and dehydrated bakery product (1) were, respectively, $1{\cdot}55 \pm 0{\cdot}18$, $7{\cdot}59 \pm 0{\cdot}08$, $2{\cdot}35$ and $2{\cdot}39$ g kg^{-1}, all higher than ingredient composition table values. Choline contents lower than table values were found in samples of poultry by-products (7) and meat and bone meal (6): $2{\cdot}18 \pm 0{\cdot}87$ and $1{\cdot}08 \pm 0{\cdot}29$ g kg^{-1}, respectively. The average choline concentration found in samples of dehulled soybean meal (7) was $2{\cdot}73 \pm 0{\cdot}18$ g kg^{-1}, similar to table values. The choline in samples of poultry fat (2) averaged $0{\cdot}48 \pm 0{\cdot}02$ g kg^{-1}. Significant correlations between the concentrations of choline and of some components of the proximate analysis were found. The analysed choline concentrations in mixed feeds were only 1·4% lower than the calculated levels based on the ingredient analyses. The procedure was adequate for choline determination in ingredients or mixed feeds. The high variability in the choline content of some ingredients may require analysis for proper feed formulation. © 1998 Society of Chemical Industry.

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Key words: choline; feed ingredients; feeds; enzymatic assay

INTRODUCTION

Reports on the choline content of feed ingredients have been found in the literature for more than 50 years. In the early reports of Engel (1942, 1943) and Rhian *et al* (1943), as well in others that followed (Almquist and Maurer 1951; Lim and Schall 1964; Lipstein *et al* 1977), the analytical procedure consisted of variations of the chemical method referred to as the reineckate technique. Horowitz and Beadle (1943) determined choline by means of a microbiological method using a mutant of *Neurospora*. Chemical and microbiological methods were compared to a chick growth assay in evaluating the choline content of some feed ingredients (Fritz *et al* 1967). A limited number of ingredients were analysed in these studies. The analytical procedures for choline have been, until recently, described as laborious, imprecise and relatively unspecific (Anonymous 1994). Menten and Pesti (1998) presented a methodology for a more rapid and precise determination of choline in feeds using an enzymatic assay.

Recent information on the choline content of feed ingredients has not been found in the scientific literature. However, all feed composition tables available to animal nutritionists display the choline content of a large number of feedstuffs. These values are probably derived from unpublished results of independent laboratories, and the methods of analyses are not reported.

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Furthermore, the feed composition tables do not provide information on the variability in choline content that can be expected for each ingredient. Differences in the origin or processing method of some feedstuffs may result in different choline contents.

Choline is an essential nutrient which is often supplemented to animal diets to improve performance, because the main ingredients may not meet minimum specifications. In order to supplement adequate amounts of choline to feeds, it is necessary to properly estimate its concentration in the ingredients. The purpose of this report is to provide information on the levels and variability in the choline content in some commonly used feed ingredients. In addition, the calculated and analysed values of some commercial poultry feeds were compared to determine the additivity of results.

EXPERIMENTAL

Choline determinations were carried out on a series of feed ingredients and mixed feeds collected from seven feed mills operated by a private company (ConAgra Poultry Company) in the US. Seven samples each of maize, dehulled soybean meal and poultry by-products (ground, rendered, clean parts of the carcass of slaughtered poultry excluding feathers), six samples of meat and bone meal (rendered mammal tissues except blood, hair, hoof, horn and hide trimmings), three samples of canola meal, two samples of poultry fat, one sample of wheat middlings, one sample of dehydrated bakery product and nine samples of broiler and broiler breeder feeds containing the above ingredients were included in the study. In addition to the choline determinations, the feed ingredients were also analysed for moisture, crude protein (CP), ash, ether extract (EE) and crude fibre (CF) according to the AOAC (1990).

The feed samples were milled using a 2-mm screen prior to the analyses. The analytical procedure previously described (Menten and Pesti 1998), using a 'hot' extraction in methanol–KOH and an enzymatic assay of choline, was utilized. Duplicate samples (weighing approximately 2 g each) were extracted in a Goldfisch apparatus. The biochemical components of the enzymatic assay were all purchased from Sigma Chemical Co (St Louis, MO, USA). The dilution factors (F) were 5 for soybean meal and poultry fat, 10 for maize, poultry by-products, meat and bone meal, wheat middlings, bakery product and the mixed feeds and 20 for the canola meal.

Correlation analyses involving the components of the proximate analysis and choline were performed (SAS 1985) for the materials with 6 or more samples.

The analysed choline values in the mixed feeds were compared, in a blind procedure, to the calculated values based on the contributions of each ingredient and supplemental choline.

RESULTS AND DISCUSSION

The determined choline concentrations in maize obtained with the present procedure (Table 1) were

TABLE 1
Choline concentration (g kg^{-1})[a] and proximate analyses of samples of maize

Sample	*Choline*	*Ash*	*Crude protein (CP)*	*Ether extract (EE)*	*Crude fibre (CF)*
1	1·74 ± 0·02	13·8	80·1	33·5	17·6
2	1·70 ± 0	12·2	80·7	32·2	17·4
3	1·35 ± 0·11	12·8	76·6	29·7	19·2
4	1·39 ± 0·04	12·3	75·4	27·9	17·4
5	1·36 ± 0·13	12·3	77·8	34·4	18·4
6	1·58 ± 0	13·1	78·4	36·5	20·9
7	1·74 ± 0·01	12·2	78·4	33·1	19·0
Average	1·55 ± 0·18	12·7 ± 0·6	78·2 ± 1·8	32·5 ± 2·9	18·6 ± 1·3
Correlation analysis[b]					
Choline		0·573	0·025	0·343	0·863
Ash	0·260		0·529	0·485	0·699
CP	0·815	0·289		0·170	0·776
EE	0·424	0·319	0·582		0·195
CF	−0·081	0·180	−0·133	0·555	

[a] Mean ± standard deviation.
[b] Values below the diagonal are correlation coefficients, and values above the diagonal are the probability levels.

more than twice as large as the values of 0·50 g kg^{-1} (NRC 1988) or 0·62 g kg^{-1} (NRC 1994) found in tables of feed composition. In the early reports, in which the reineckate procedure was used, the choline in maize ranged from 0·32 to 0·60 g kg^{-1} (Engel 1943; Rhian *et al* 1943, Almquist and Maurer 1951). Higher values of choline in maize (1·30 g kg^{-1}), closer to those obtained in the present study, have been reported only by Lim and Schall (1964). The microbiological method of Horowitz and Beadle (1943) yielded a choline concentration in maize of 0·34 g kg^{-1}. When the extraction step was carried out using the 'cool' (thimble) method, the present authors also found choline values of 0·50–0·53 g kg^{-1} in maize (Menten and Pesti 1998).

The variability in choline content among the maize samples (coefficient of variation, CV = 11·6%) was slightly greater than for the components of the proximate analysis studied (ash, CP, EE, CF), which had CVs ranging from 2·3 to 8·9% (Table 1). The correlation analysis indicated a positive significant correlation ($r = 0{\cdot}807$, $P = 0{\cdot}025$) between the choline and the CP contents of the maize samples; no significant correlations involving choline and the other components were detected ($P > 0{\cdot}05$).

The average choline concentration in dehulled soybean meal was identical to the NRC (1988, 1994) value of 2·73 g kg^{-1}, but a CV of 6·6% was found in the samples analysed (Table 2). Using the 'cool' thimble extraction and the reineckate quantification of choline, concentrations of 2·99 g kg^{-1} (Engel 1943), 2·80 and 3·40 g kg^{-1} (Rhian *et al* 1943), 2·84 and 2·87 g kg^{-1} (Almquist and Maurer 1951) and 1·86 g kg^{-1} (Fritz *et al* 1967) have been reported. The microbiological assay of choline in dehulled soybean meal has produced results as variable as 1·80, 3·48 and 6·65 g kg^{-1} (Fritz *et al* 1967; Molitoris and Baker 1976). The enzymatic assay of choline extracted through the 'cool' technique resulted in choline values of 2·04 to 2·35 g kg^{-1}, while the present 'hot' extraction yielded 2·58 g kg^{-1} choline (Menten and Pesti 1998).

The concentration of choline in soybean meal was positively correlated ($r = 0{\cdot}800$, $P = 0{\cdot}031$) with the EE in the samples (Table 2), indicating that variations in the processing for oil extraction of the soybeans is a factor influencing the choline content of the soybean meal. There was also a negative association between the choline and CF contents ($r = -0{\cdot}752$, $P = 0{\cdot}051$) of the soybean meal samples. The variability of EE and CF in these samples was higher than for choline (CV = 40·3% for EE, CV = 15·1% for CF); thus, these components of the proximate analysis may be useful in estimating the choline content of soybean meal.

The choline content of the different poultry by-products analysed was highly variable (Table 3); this probably reflects the large differences in choline concentration between the hydrolysed feather meal (0·89 g kg^{-1}) and the poultry by-product meal (5·95 g kg^{-1}) values published by the NRC (1994). Because the proportions of the components used in manufacturing each product and the processing methods were likely to be very different between plants, the variation found in the choline content of the poultry by-products was not unexpected. Likewise, the proximate composition of the samples was highly variable

TABLE 2

Choline concentration (g kg^{-1})[a] and proximate analyses of samples of dehulled soybean meal

Sample	*Choline*	*Ash*	*CP*	*EE*	*CF*
1	2·83 ± 0·04	64·9	489·6	18·1	31·0
2	3·05 ± 0·02	65·8	489·1	17·9	33·1
3	2·70 ± 0·02	59·9	466·8	12·3	40·2
4	2·48 ± 0·04	61·3	483·0	4·0	48·0
5	2·73 ± 0·01	62·6	478·2	15·8	37·0
6	2·70 ± 0·07	65·9	480·6	8·0	35·7
7	2·65 ± 0·04	69·0	484·3	14·0	34·4
Average	2·73 ± 0·18	64·2 ± 3·1	481·7 ± 7·8	12·9 ± 5·2	37·1 ± 5·6
Correlation analysis[b]					
Choline		0·500	0·384	0·031	0·051
Ash	0·309		0·117	0·471	0·099
CP	0·392	0·646		0·512	0·326
EE	0·800	0·329	0·301		0·022
CF	−0·752	−0·670	−0·438	−0·828	

[a] Mean ± standard deviation.
[b] Values below the diagonal are correlation coefficients, and values above the diagonal are the probability levels.

TABLE 3
Choline concentration (g kg^{-1})[a] and proximate analyses of samples of poultry by-products

Sample[b]	*Choline*	*Ash*	*CP*	*EE*	*CF*
1	3·32 ± 0·02	175·9	626·3	106·1	13·8
2	3·12 ± 0·01	203·7	605·7	113·4	18·4
3	2·47 ± 0·11	175·1	638·1	111·4	12·7
4	1·87 ± 0·04	83·5	583·9	229·7	7·0
5	1·73 ± 0·07	117·3	609·8	209·1	4·2
6	1·97 ± 0·13	50·7	486·1	379·4	4·1
7	0·78 ± 0·02	23·9	752·8	113·5	7·2
Average	2·18 ± 0·87	118·6 ± 69·0	614·7 ± 78·9	180·4 ± 101·7	9·6 ± 5·4
Correlation analysis[c]					
Choline		0·008	0·444	0·561	0·046
Ash	0·885		0·894	0·209	0·021
CP	−0·348	−0·062		0·032	0·660
EE	−0·268	−0·542	−0·797		0·077
CF	0·762	0·830	0·204	−0·704	

[a] Mean ± standard deviation.
[b] Samples 1–3, poultry by-product meal; samples 4–5, pressed poultry meal; sample 6, poultry conglomerate; sample 7, sludge-feather meal blend.
[c] Values below the diagonal are correlation coefficients, and values above the diagonal are the probability levels.

(CV = 56·2–8·2% for ash, EE and CF). However, even the products whose composition was closer to that of a typical poultry by-product meal (samples 1–3, Table 3) did not have choline contents that approached the table value.

The higher choline values in the poultry by-products were associated with higher contents of ash ($r = 0{\cdot}885$, $P = 0{\cdot}008$) and of CF ($r = 0{\cdot}762$, $P = 0{\cdot}046$). These correlations indicate that ash and CF may be important in screening poultry by-products for choline content and that the ash-rich and CF-rich tissues (especially bones) are the main sources of choline in poultry by-products.

There was also considerable variation in the choline content (CV = 26·8%) among the samples of meat and bone meal analysed (Table 4). The average value was

TABLE 4
Choline concentration (g kg^{-1})[a] and proximate analyses of samples of meat and bone meal

Sample	*Choline*	*Ash*	*CP*	*EE*	*CF*
1	1·03 ± 0·01	328·8	516·8	82·1	21·9
2	1·04 ± 0·03	330·4	523·9	76·8	23·0
3	1·07 ± 0·01	326·1	515·7	78·0	23·3
4	1·17 ± 0·04	313·1	509·4	91·2	24·9
5	·64 ± 0·01	400·3	445·0	74·2	22·7
6	1·53 ± 0·02	298·5	484·9	116·7	25·5
Average	1·08 ± 0·29	332·9 ± 35·2	499·3 ± 29·8	86·5 ± 15·9	23·5 ± 1·4
Correlation analysis[b]					
Choline		0·007	0·438	0·022	0·089
Ash	− 0·930		0·130	0·151	0·223
CP	0·395	− 0·689		0·890	0·892
EE	0·877	− 0·664	− 0·073		0·043
CF	0·745	−0·585	−0·072	0·826	

[a] Mean ± standard deviation.
[b] Values below the diagonal are correlation coefficients, and values above the diagonal are the probability levels.

TABLE 5
Choline concentration in samples of canola meal, poultry fat, wheat middlings and dehydrated bakery product (g kg^{-1})[a]

Ingredient	*Sample*	*Choline*
Canola meal	1	7·52 ± 0·10
	2	7·58 ± 0·25
	3	7·68 ± 0·04
	Average	7·59 ± 0·08
Poultry fat	1	0·46 ± 0·03
	2	0·49 ± 0·01
	Average	0·48 ± 0·02
Wheat middlings	1	2·35 ± 0·01
Bakery product	1	2·39 ± 0·11

[a] Mean ± standard deviation.

only about half the amount of choline reported for meat and bone meal in feed composition tables (2·14 g kg^{-1} (NRC 1988) and 2·00 g kg^{-1} (NRC 1994)). These samples were not atypical in other respects; samples 1–4 had proximate compositions (510–520 g kg^{-1} CP, 80–90 g kg^{-1} EE) similar to table values for meat and bone meal. Although the CVs for ash and EE content (10·6 and 18·4%, respectively) were lower than for choline (Table 4), the choline concentration was negatively correlated with the ash content ($r = -0{\cdot}930$, $P = 0{\cdot}007$) and positively correlated with the EE ($r = 0{\cdot}877$, $P = 0{\cdot}022$) in the samples analysed. This indicates that the inclusion of bones in meat and bone meal lowers the choline levels, while the inclusion of fat-containing tissues increases the levels of choline in the product.

The choline concentration of the remaining feed ingredients analysed are presented in Table 5. The samples of canola meal had very little variability in their choline content (CV = 1·0%) compared to the other vegetable sources, maize and soybean meal. However, the average choline concentration was much higher than the 6·70 g kg^{-1} found in the NRC (1988, 1994) tables. The choline content in three samples of canola meal analysed by the reineckate technique ranged from 4·65 to 5·72 g kg^{-1} (March and MacMillan 1980). In the present authors' previous study (Menten and Pesti 1998), the 'cool' extraction of choline from a canola meal sample also yielded lower values than the 'hot' extraction, used in the present study. The sample of wheat middlings (186·7 g kg^{-1} CP, 76·3 g kg^{-1} CF) had a higher choline content than the table values (1·25 g kg^{-1} (NRC 1988) and 1·44 g kg^{-1} (NRC 1994)). Likewise, the dehydrated bakery product (106·7 g kg^{-1} CP, 122·2 g kg^{-1} EE) was also higher in choline than the NRC (1988, 1994) table value of 0·92 g kg^{-1}. The choline content of the two samples of poultry fat, produced in different rendering plants, was similar; however, no published values have been found for comparison.

Nine samples of mixed poultry feeds, which contained all the ingredients presented in Tables 1–5 , were also analysed for choline. These results, as well as the calculated choline contents, are presented in Table 6. It was found that the analysed choline and the calculated value based on the ingredient analyses generally reached a good agreement. The deviations between analysed and calculated choline in the individual feeds varied from −8·6 to 16·1%; however, on average, the difference was only −1·4%. The deviations observed can be attributed

TABLE 6
Choline content of mixed poultry feeds: analytical results and calculated values based on the choline content of the ingredients

Feed	*Analysed choline (AC) (g kg^{-1})*	*Calculated choline (g kg^{-1})*		*AC-IA*	
		IA[a]	*NRC*[b]	*(g kg^{-1})*	*% Difference*[c]
1. Pre-starter	2·46 ± 0·09	2·44	2·04	0·02	0·08
2. Starter	2·37 ± 0·06	2·50	2·09	− 0·13	− 5·2
3. Starter	2·13 ± 0·07	2·50	1·99	− 0·37	− 14·8
4. Grower	1·86 ± 0·07	2·03	1·71	− 0·17	− 8·4
5. Grower	2·47 ± 0·14	2·38	1·86	0·09	3·8
6. Withdrawal	2·13 ± 0·02	1·98	1·77	0·15	7·6
7. Withdrawal	1·87 ± 0·03	1·91	1·45	− 0·04	− 2·1
8. Breeder	1·88 ± 0·16	1·84	1·39	0·04	2·2
9. Breeder	2·09 ± 0·09	1·92	1·54	0·17	8·8
Average	2·14 ± 0·25	2·17 ± 0·28	1·76 ± 0·26	− 0·03	− 1·4

[a] Ingredient analysis based on analysed values of choline in the feed ingredients and choline chloride supplementation.
[b] Based on choline content of feed found in the NRC (1994) composition table.
[c] 100 (AC-IA)/IA.

to several factors, such as improper mixing of choline at the feed mill, inadequate sampling of the feed for analysis or errors in the analytical procedure. Although the analysed choline was higher than the table value for some ingredients and lower for others, the calculated choline based on ingredient analyses was 23% greater than calculating based on the values found in the NRC (1994) table.

CONCLUSIONS

The concentrations of choline in feed ingredients obtained with the analytical procedure used in this study were higher than the values found in tables of feed composition for maize, canola meal, wheat middlings and dehydrated bakery product, lower for poultry by-products and meat and bone meal and similar for dehulled soybean meal. Because of these differences, the adoption of the present procedure for choline analysis would require a re-evaluation of the choline content of a number of other feed ingredients and a re-evaluation of the choline requirements of animals.

The elevated variability in choline content encountered among samples of poultry by-products and meat and bone meal may warrant routine analysis for choline and/or a better standardisation of the products used in feed formulation. Even for the major constituents of a feed, maize and soybean meal, which has only a moderate variability in the samples analysed, the analysis of choline may be advantageous. If, for example, the maize and soybean meal samples with the highest and the lowest concentrations of choline (Tables 1 and 2) were combined in poultry feeds ($620\ g\ kg^{-1}$ maize, $330\ g\ kg^{-1}$ soybean meal), the difference in choline concentration between the two feeds would be $0{\cdot}43\ g\ kg^{-1}$. This difference in choline supplementation is important in terms of feed cost and performance of the animals.

Interesting correlations between the concentrations of choline and several components of the proximate analysis have been detected. Although the number of samples of each ingredient was not large, those correlations may prove to be helpful in screening products with different choline contents based on the proximate analysis. It may also be possible to estimate choline from the results of the proximate analysis.

The determined choline in the mixed feeds and the calculated choline based on ingredient analyses reached an acceptable agreement. Thus, the analytical procedure utilised was equally adequate for mixed feeds and feed ingredients ranging from cereal grain to rendering products.

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REFERENCES

Almquist H J, Maurer S 1951 Choline content of certain feedstuffs. *Poultry Sci* **30** 789–790.

Anonymous 1994 *Choline, Functions and Requirements.* DuCoa, Highland Park, IL, USA, pp 36–39.

AOAC 1990 *Official Methods of Analysis* (15th edn). Association of Official Agricultural Chemists, Washington, DC, USA.

Engel R W 1942 Modified methods for the chemical and biological determination of choline. *J Biol Chem* **144** 701–710.

Engel R W 1943 The choline content of animal and plant products. *J Nutr* **25** 441–446.

Fritz J C, Roberts T, Boehne J W 1967 The chick's response to choline and its application to an assay for choline in feedstuffs. *Poultry Sci* **46** 1447–1453.

Horowitz N H, Beadle G W 1943 A microbiological method for the determination of choline by use of a mutant of neurospora. *J Biol Chem* **150** 325–333.

Lim F, Schall E D 1964 Determination of choline in feeds. *J Assoc Off Agric Chem* **47** 501–503.

Lipstein B, Bornstein S, Budowski P 1977 Utilization of choline from crude soybean lecithin by chicks. 1. Growth and prevention of perosis. *Poultry Sci* **56** 331–336.

March B E, MacMillan C 1980 Choline concentration and availability in rapeseed meal. *Poultry Sci* **59** 611–615.

Menten J F M, Pesti G M 1998 The determination of the choline content of feed ingredients using choline kinase. *J Sci Food Agric* **78** 395–398.

Molitoris B A, Baker D H 1976 Assessment of the quantity of biologically available choline in soybean meal. *J Anim Sci* **42** 481–489.

NRC 1988 *Nutrient Requirements of Swine* (9th edn). National Research Council National Academy Press, Washington, DC, USA, pp 56–57.

NRC 1994 *Nutrient Requirements of Poultry* (9th ed). National Research Council National Academy Press, Washington, DC, USA, pp 62–65.

Rhian M, Evans R J, St John J L 1943 The choline content of feeds. *J Nutr* **25** 1–5.

SAS 1985 *SAS User's Guide: Statistical Version* (5th edn). SAS Institute, Cary, NC, USA.