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THE TRYPTOPHAN CONTENT OF PEARL MILLET GRAINS AS A FUNCTION OF NITROGEN CONTENT

JACQUES LANDRY and SONIA DELHAYE

INRA, Centre de Biotechnologie Agro-Industrielle, Laboratoire de Chimie biologique, F-78850 Thiverval-Grignon, France

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Key Word Index—*Pennisetum thyphoïdes*; *Pennisetum americanum*; Gramineae; pearl millet; grain; tryptophan; nitrogen.

Abstract—The tryptophan content of pearl millet (*Pennisetum thyphoïdes* or *P. americanum*) was determined for 17 samples, using a procedure that allowed the true quantity of this amino acid to be evaluated. The linear relationship found between the level of tryptophan in the dry matter and the nitrogen content of the grain was compared with the homologous ones published for foxtail millet and maize. The relationships for pearl and foxtail millets were characterized by close, if not identical, parameters and by positive *y*-intercepts, indicative of higher tryptophan content in prolamins (2.4%) than in non-prolamin (1.2–1.4%) proteins. Comparatively, the slope of the relationship determined for maize was one-fifth of that for millets and the *y*-intercept was negative. A variability in the level of tryptophan in grain proteins was evidenced when this parameter was regressed against the reciprocal of nitrogen content.

INTRODUCTION

The production of millets is the least important among the eight cereal crops in the world. Pearl millet (*Pennisetum thypoïdes*, or *P. americanum*) is the most cultivated species, mainly in the semi-arid areas of Africa and South Asia where it constitutes a component part of the diet [1]. Despite this use, the tryptophan content of grains is not known with accuracy. Swaminathan *et al.* [2] reported tryptophan contents ranging from 0.7 to 1.7% (g 100 g^{-1} protein) for nitrogen contents varying from 1.3 to 3.2%. In the same paper these workers indicated tryptophan contents of foxtail millet (*Setaria italica*) amounting to 0.8 and 1.1% for respective nitrogen per cents of 1.6 and 2%, whereas Mossé *et al.* [3] analysing 13 samples found tryptophan ranging from 1.7 to 2% when nitrogen varied from 1.8 to 3.6%. According to Ejeta *et al.* [4], the tryptophan level in pearl millet was probably comparable with that found in high-lysine sorghum on the basis of a calculated value from its protein fractions, i.e. *ca* 1% tryptophan for 1.7% nitrogen. A similar level can be calculated from the data of Chandna and Matta [5]. However, there are still uncertainties regarding the tryptophan level of protein fractions, especially prolamins: 1.2% after Nwasike *et al.* [6], using the procedure of Villegas and Mertz [7], 0.40–0.66% after Chandna and Matta [5] using the method of Spies and Chambers [8], and 3% after Sainani *et al.* [9], using the spectrophotometric assay of Bencze and Schmidt [10]. This latter value agrees with the old value (2.8%) reported by Sawhney and Naik [11] in 1969, using the procedure in ref. [8], and by Narayamurti and Aiyar [12] in 1930 for pearl millet extracted with 70% ethanol. The present study was aimed to remove these uncertainties by determining the tryptophan content of 17 samples of pearl millet, using a reliable procedure for the quantification of this amino acid.

RESULTS AND DISCUSSION

Table 1 gives the nitrogen content $[N]_{DM}$ (expressed as g 100 g^{-1} dry matter) and the concentration of Trp in protein $[Trp]_P$ (expressed as g 100 $g^{-1} \equiv$ g 16 g^{-1} N) for the 17 samples analysed. $[N]_{DM}$ ranged from 1.20 to 3.7% and $[Trp]_P$ from 1.49 to 2.9%. There was a slight tendency for $[Trp]_P$ to increase with $[N]_{DM}$ increasing. The values of $[Trp]_P$ found for low values of $[N]_{DM}$ were higher than those reported by Swaminathan *et al.* [2] for a similar value of $[N]_{DM}$.

Figure 1 depicts variations in tryptophan content in dry matter $[Trp]_{DM}$ plotted against $[N]_{DM}$. A straight line was obtained indicating that the amount of tryptophan in dry matter increased linearly with the accumulation of protein. The line of best fit through the data was calculated by the method of least squares. For the sake of comparison data, tryptophan values and regression line concerning foxtail millet published by Mossé *et al.* [3] are also shown. Regarding maize, which belongs to the same tribe (Panicoideae) as does millet and contributes also to the diet of people eating millet, only the regression line from data reported by Landry and Delhaye [13] was

Table 1. Nitrogen and tryptophan contents of 17 pearl millet samples*†

Sample	$[N]_{DM}$	$[Trp]_P$	Sample	$[N]_{DM}$	$[Trp]_P$
1	1.20	1.49	10	2.22	1.84
2	1.41	1.49	11	2.73	1.79
3	1.50	1.65	12	2.93	1.89
4	1.63	1.82	13	3.10	1.82
5	1.75	1.65	14	3.20	1.81
6	1.87	1.91	15	3.29	1.73
7	1.93	1.68	16	3.34	1.98
8	1.96	1.68	17	3.70	2.01
9	2.03	1.65			

*$[N]_{DM}$ is expressed as gN. $100\,g^{-1}$DM, $[Trp]_P$ as gTrp. $100\,g^{-1}$ protein or $16\,g^{-1}$N.

†Values are the average of two determinations.

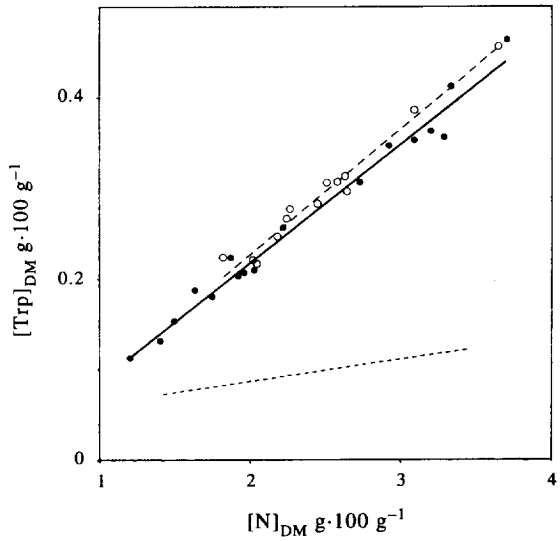

Fig. 1. Relationships between tryptophan and nitrogen contents of millet and maize grains. ●—● Experimental points and regression line for pearl millet (present work); ○--○ Experimental points and regression line for foxtail millet (from data of ref. [3]); --- regression line for maize (from data of ref. [13]).

graphed. Regression lines $[Trp]_{DM} = a[N]_{DM} + b$ pertaining to pearl and foxtail millets were very close, if not identical, but markedly different from that relative to maize.

The similarities and differences depicted in Fig. 1 were better assessed from the data presented in Table 2, which gives the statistical parameters for the regression lines. Pearl and foxtail millets were characterized by regression lines with similar coefficients of determination, slopes and negative y-intercepts. The regression line for maize differed from previous ones by a lower coefficient of determination, probably related to a higher number of grain samples, a five times lower slope and a positive y-intercept. The negative y-intercept indicated a tryptophan content higher for prolamins or storage proteins than for non-prolamin proteins or basal proteins [14, 15]. The tryptophan content of salt-soluble proteins, prolamins and glutelins isolated by sequential extraction of defatted ground grains originated from one pearl millet sample was evaluated to 1.15, 2.40 and 1.42 g $16\,g^{-1}$ N, respectively. On the other hand, a correction factor of 1.1 should be applied to the data reported for the tryptophan of foxtail millet by Mossé *et al.* [3] since an underestimation averaging 10% has been shown to occur when regression lines [Trp] vs $[N]_{DM}$ determined for wheat [16], maize [17] and barley [18] by these workers were compared with the homologous ones obtained by Landry and Delhaye [13]. Actually, such a correction was not found to be necessary, since tryptophan of samples of pearl and foxtail millet was assayed by one of the present authors, using a procedure (transfer of sample–barita mixtures, subjected to hydrolysis in the autoclave when water was boiling) leading to a quantitative recovery of tryptophan. Consequently, the tryptophan contents of grains of pearl and foxtail millets can be considered as similar for a given nitrogen content.

Table 2. Statistical parameters concerning the relationship $[Trp]_{DM} = a[N]_{DM} + b$ and $[Trp]_P = a' + b'[N]_{DM}^{-1}$ for pearl millet, foxtail millet and maize*

parameter	Pearl millet	Foxtail millet†	Maize‡
a(SE)§	0.131 (0.005)	0.138 (0.007)	0.0248 (0.0017)
b(SE)	−0.045 (0.011)	−0.049 (0.017)	0.0371 (0.0031)
r_A^2	0.981	0.980	0.845
a'(SE)	2.094 (0.076)	2.154 (0.141)	0.456 (0.023)
$a'/16$	0.131	0.135	0.0285
b'(SE)	−0.702 (0.150)	−0.655 (0.333)	0.488 (0.064)
$b'/16$	−0.044	−0.041	0.0305
r_B^2	0.594	0.260	0.605

*$[Trp]_{DM}$ and $[N]_{DM}$ are expressed as g. $100\,g^{-1}$ DM, $[Trp]_P$ as gTrp. $100\,g^{-1}$ protein or $16\,g^{-1}$N.

†From data of ref. [3].

‡From data of ref. [13].

§Standard error of estimate.

Introducing the tryptophan content in protein $[Trp]_P = 16[Trp]_{DM} \cdot [N]_{DM}^{-1}$ into the relationship $[Trp]_{DM} = a[N]_{DM} + b$ leads to

$$[Trp]_P = 16a + 16b[N]_{DM}^{-1} = a' + b'[N]_{DM}^{-1},$$

indicating that the variations of $[Trp]_P$ vs $[N]_{DM}$ fitted a hyperbolic function if b was significantly different from 0. Furthermore, the variations of $[Trp]_P$ plotted against the changes of the reciprocal of $[N]_{DM}$ must be linear. The parameters of such a regression line calculated by the method of least squares are presented in Table 2, together with those related to the homologous lines obtained with foxtail millet and maize. Comparison of the data given in Table 2 emphasizes the great similarity between a and $a'/16$ or between b and $b'/16$, as well as the difference in r^2. So, only 60% of variations of protein tryptophan recorded for pearl millet and maize, and 26% of those observed for foxtail against the changes of $[N]_{DM}$, can be explained by a hyperbolic relationship. In this latter case b' was not significantly different from zero, indicating the independence of $[Trp]_P$ from $[N]_{DM}$. The same should be true for the level of prolamin in protein towards $[N]_{DM}$, which was in disagreement with the data obtained with pearl millet. Actually, the removal of the two values ($[N]_{DM} = 1.82\%$; $[Trp]_P = 1.95\%$) from the data reported for foxtail millet by Mossé *et al.* [3] led to the following regression:

$$[Trp]_P = (2.335 \pm 0.125) - (1.130 \pm 0.303)[N]_{DM}^{-1}$$
$$r_B^2 = 0.581,$$

which displayed characteristics coherent with those found for pearl millet.

The parameter a' corresponds to the mean content in tryptophan present in the proteins accumulated in the dry matter of grains, as assessed by the increase of $[N]_{DM}$. Since these proteins were made up of a mixture of prolamins and non-prolamins, respectively, rich and poor in tryptophan, prolamins of pearl millet should have a tryptophan content higher than 2.1 g 16 g^{-1} N. As mentioned above, this content was evaluated to 2.4 g 16 g^{-1} N for proteins extracted by 55% isopropanol + 1% 2-mercaptoethanol, and to 2.8–3.0 g 16 g^{-1} N for proteins extracted by 70% ethanol without reductant [9, 11, 12]. These discrepancies suggested differences in the exractability and in the tryptophan level of various polypeptides constituting alcohol-soluble proteins of pearl millet.

The low values of coefficients of determination r_B^2 (Table 2) indicated variability in the tryptophan content of millets. This, shown with 12 or 17 samples, was of the same order of magnitude to that observed with 40 samples of maize. It could be a reflection of variable levels of prolamins for a given content of nitrogen. Such a variability can also be seen when $[Trp]_{DM}$ is regressed against $[N]_{DM}$, but it is concealed by the high values of coefficients of a perfect relationship between $[Trp]_{DM}$ and $[N]_{DM}$ [3, 16–18]. The high values of correlation coefficients obtained in that case are related to the occurrence of $[N]_{DM}$ in both sides of the equation connecting $[Trp]_{DM} (\equiv [Trp]_P \cdot [N]_{DM}/16)$ with $[N]_{DM}$.

Finally, the data presented in this study, when compared with those reported in the literature, point out the lack of reliability of colorimetric procedures for assaying tryptophan of protein extracts. They contribute direct, as well as indirect evidence, that pennisetin, the millet prolamin, contains an appreciable amount of tryptophan contrary to maize zein which is devoid of this amino acid.

EXPERIMENTAL

The 17 samples studied corresponded to varieties collected by ORSTOM and were provided by the Laboratoire de Génétique et de Physiologie du Développement, CNRS, 91190 Gif sur Yvette, France. Grains were finely ground in a Foss Electric mill. The dry matter was determined by heating meals at 105° for 20 hr. Total N was assayed in duplicate by a Kjeldahl procedure.

Protein frs were extracted from meal defatted with Me_2CO at −10° using, successively, 0.5 NaCl, H_2O and 55% *i*-PrOH containing 1% 2-mercaptoethanol according to Landry and Moureaux [19]. Albumins and globulins were isolated after dialysis of a salt extract against H_2O. Residual proteins obtained after alcoholic extraction were considered as glutelins.

Tryptophan was analysed in duplicate according to Delhaye and Landry [20]. It is worth recalling that the transfer of tubes containing the mixts subjected to barytic hydrolysis to a bench-top autoclave when H_2O was boiling is a prerequisite for obtaining quantitative results.

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