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By

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In studying animal feeding, it is of primary importance to investigate the details of the metabolism of lysine, one of the essential amino acids. Formerly, Osborne and Mendel (1) have observed that lysine is essential to the growth of albino rats. It is not indispensable to the maintenance of the bodies of livestock, but it has been contended that it affects their products, and that the limiting factor of milk production in dairy cows might be the content of lysine in feeds, rather than of crude protein, as has been generally accepted (2). This contention, however, lacks adequate experimental proof.

We have undertaken to clarify this problem, to the end of establishing a theoretical feeding standard. First of all, we must point out that the hitherto available method of quantitative analysis for lysine was rather complicated in operation and incapable of obtaining adequately precise results. For example, the method proposed by Kossel (3), Block (4), and Van Slyke (5) requires the preliminary separation of other coexisting amino acids, either for estimating the residual lysine or for deducing the quantity of lysine from the estimated quantity of other amino acids. Kurtz (6) has proceeded by separating lysine as benzoyl lysine copper directly from protein hydrolysates, but this method has the defect being inapplicable to certain protein. Turba (7) also succeeded in separating the copper salt of benzal lysine from casein and gelatin, but the authors could not obtain the same results from the immediate hydrolysates of feeds. Kibrick's colorimetry (8) is not yet ripe for obtaining adequate results with the hydrolysates of feeds. Since we have not succeeded in isolating a strain with a specific decarboxylase activity towards lysine, the decarboxylase method employing the organisms which we have isolated could not be applied to the estimation of lysine content in such hydrolysates.

At this juncture, we had the luck of receiving the kind donation of Bacterium

cadaveris (National Collection of Type Cultures ref. no. 6578) producing 1(+)-lysine decarboxylase from Dr. E. F. Gale of Cambridge University, who has established the method of determining the quantity of 1(+)-lysine by the use of its decarboxylase (9, 10, 11). With this, we have been enable to determine the lysine content in various feeds and, in particular, to estimate the effects of defatting of rice refuse and evaporation of its hydrolysates upon the lysine content and the recovery rate of lysine by the addition test.

Materials and Methods

Lysine Decarboxylase Preparation

The specific lysine decarboxylase preparation for estimation of 1(+)-lysine used in these experiments corresponds to the acetone powder from Bact. cadaveris no. 6578 described by Gale (9).

Bact. cadaveris is grown for 24 hr at 25°C in a medium containing 3% tryptic digest of casein and 2% glucose and is made into acetone powder (10). Freshly prepared acetone powder frequently showed a small decarboxylase activity towards arginine, but upon keeping it in the ice chest for a few days, the arginine enzyme lost its activity, while the lysine decarboxylase remained active for several weeks (9). This powder was rubbed up in M/5 phosphate buffer pH 6.0 (20 mg. powder / cc buffer) and 0.5 cc of the suspension was used for each estimation.

Feed Hydrolysates

Feeds on the market in Sendai'rice refuse and wheat bran, ground wheat, white dent corn, soybean oil meal and fish meal were used for the experiments. The chemical composition of these feeds is shown in Table 1. As these samples were always used in powder after dehydration, the total nitrogen values calculated for dry and defatted materials were also shown in the Table, and in the case of rice refuse, the measured value obtained from defatted and dehydrated material was also inserted. The latter is lower by 5.4 % than the corresponding value obtained by the above computation, and the discrepancy is presumably due to loss in defatting.

The hydrolysates of feeds were prepared, in all cases, by the accurate weighing of approximately 3–6 g. feed of known chemical composition, followed by hydrolysis, adding 10 times 20 % HCl in feed weight. And then the hydrolysis were completed with a reflux condensor, in 24 hr with soybean oil meal and fish meal, and in 12 hr with the other samples. After hydrolysis, the hydrolysates were filtered, thoroughly washed with hot water and then the filtrate and the washings were evaporated together to dryness in vacuo. The free HCl present therein is eliminated by evaporation in vacuo, repeating the addition of water five times. Then the dry residue was dissolved in about 15 cc

Table 1. Chemical composition of feeds used (%)

	Rice refuse	Wheat bran	Ground wheat	White dent corn	Soybean oil meal	Fish meal
Moisture	14.79	13.05	13.73	13.72	17.18	12.40
Crude protein	9.94	15.75	12.56	9.31	38.69	64.25
Crude fat	19.20	3.97	1.40	3.79	5.28	4.76
Nitrogen free extract	35.94	53.41	67.66	69.89	30.67	2.59
Crude fiber	11.29	9.88	2.93	1.98	2.53	4.55
Ash	8.84	3.94	1.72	1.31	5.65	11.45
Total Nitrogen	1.59	2.52	2.01	1.49	6.19	10.28
Total-N/Moist. and crude fat free base	2.41 (2.28)	3.04	2.37	1.81	7.98	12.41

( ) The measured value obtained from defatted and dehydrated material.

of water, the pH of the solution adjusted to 5 with CO_2 free NaOH solution and the volume made up to 25 cc Most of the samples of the hydrolysates were condensed so as to give off 50–500 mm^3 of CO_2 per 0.5 cc.

In the case of rice refuse, samples were prepared both from raw rice refuse not defatted and from rice refuse defatted with ether in the Soxhlet extractor. One part of the hydrolysates obtained from each was subjected to evaporation in vacuo under low temparature and the other to evaporation by heating on boiling water bath.

10 cc of a ca. M/30 l(+)-lysine HCl solution (Lysine-N : 8.30 mg.) was added to defatted rice refuse before hydrolysis. The lysine content in them was measured after the former procedure. The quantity of lysine in defatted rice refuse hydrolysate measured in the foregoing test was subtracted from this content to determine the recovery of the added lysine.

Manometric Arrangement

The method depedns in this case upon the manometric measurement of the CO_2 liberated from lysine by the specific lysine decarboxylase at the optimum pH 6.0 and 30°C. Warburg manometers were used containing 0.5 or 1.0 cc of feed hydrolysate together 2.0 cc of M/5 phosphate buffer pH 6.0 in the main cup, and in the side bulb 0.5 cc enzyme preparation made up in the same buffer and the other side bulb 0.25 cc 8N-H_2SO_4.

At pH 6.0 the liberation of CO_2 was not complete. Accordingly, a calculated correction might not be accurate (10). Therefore, in order to estimate total CO_2 the "acid-tip" manometric method (12) was applied at the end of the experiment by adding 0.25 cc 8N-H_2SO_4 in the second side bulb.

The lysine content in the 5 items of feeds except rice refuse is given in Table 2, as shown in percentage of lysine-N against the total nitrogen.

Table 2. Lysine content of feeds estimated by L(+)-lysine decarboxylase.

		Wheat bran	Ground wheat	White dent corn	Soybean oil meal	Fisn meal
Total vol.	(cc.)	25	25	25	25	25
Total N	(mg.)	166.3	143.7	109.8	249.0	386.9
Experimental sample	(cc.)	0.5	0.5	1.0	0.5	0.5
CO_2 liberated	(mm^3.)	136 137 141	66 67 71	68 72 74	247 248 252	483 485 490
Mean CO_2	(mm^3.)	138	68	71.3	249	486
Corrected CO_2	(mm^3.)	141	69	73	254	496
Total CO_2	(mm^3.)	7050	3450	1825	12700	24800
Total lysine-N	(mg.)	8.81	4.31	2.28	15.88	31.00
Lysine-N as % total N		5.30	3.00	2.08	6.38	8.01

Table 3 shows the lysine content of raw and ether defatted rice refuse hydrolysates, evaporated both in vacuo and by heating.

Table 3. Lysine content of raw and defatted rice refuse hydrolysates, evaporated both in vacuo and by heating.

		raw rice refuse		ether defatted rice refuse	
		evaporation in vacuo	evaporation by heating	evaporation in vacuo	evaporation by heating
Total vol.	(cc.)	25	25	25	25
Total N	(mg.)	72.1	72.2	91.5	92.6
Experimental sample	(cc.)	0.5	0.5	0.5	0.5
CO_2 liberated	(mm^3.)	91 95 96	88 90 93	102 104 109	97 97 101
Mean CO_2	(mm^3.)	94	90.3	105	98.3
Corrected CO_2	(mm^3.)	96	92	107	100
Total CO_2	(mm^3.)	4800	4600	5350	5000
Total lysine-N	(mg.)	6.00	5.75	6.69	6.25
Lysine-N as % total N		8.32	7.96	7.31	6.75

The recovery rate of lysine added to the hydrolysate of defatted rice refuse is shown in Table 4.

Table 4. Recovery of added lysine on the defatted rice refuse after hydrolysis and evaporation

(1)	(2)	(3) (1)−(2)	(4)	(3)/(4)×100
Total Lysine-N found	Lysine-N (contained in defatted rice refuse)	Lysine-N recovered	Lysine-N added	Recovery rate
(mg.)	(mg.)	(mg.)	(mg.)	(%)
13.38	5.30	8.08	8.30	97

Discussion

Gale (13) has reported that the bacterial strains producing lysine decarboxylase are chiefly coliform organisms. Accordingly, at the outset of our experiments, we tried to obtain some strains of high lysine decarboxylase activity by isolating Bact. coli from human faeces using Endo's medium. Out of about 100 strains a strain no. 65 was isolated as the highest in lysine decarboxylase activity, but both in intact cell suspension and in acetone powder, this strain contained a considerable quantity of arginine decarboxylase, which was not eliminated by keeping the acetone powder in an ice chest for three or more days. In the so-called stage of crude extract (10), e.g., when 20 mg. of acetone powder was extracted with 1 cc of borate buffer pH 8.5, the arginine enzyme was destroyed, to be sure, but the activity of the lysine enzyme also decreased to about one third, as measured by the volume of CO_2 liberated per unit time, when 50 mg. of acetone powder was extracted with 1 cc of the same buffer, the lysine enzyme was retained by half, but the coexisitng arginine enzyme was not completely destroyed. Thus it was ascertained that this strain no. 65 of Bact. coli was not satisfactory for the determination of lysine content in feed hyrolysates, either in acetone powder or in its crude extract.

The acetone powder prepared from Bact. cadaveris could be entirely freed from arginine decarboxylase and a good specific l(+)-lysine decarboxylase preparation could be obtained.

Upon comparing the lysine contents of various feeds shown in Table 2 and 3, with the values obtained by the Van Slyke's nitrogen distribution method, given in Iwata's Japanese report (14), — 3.11% for rice refuse, 2.92% for wheat bran and 3.11% for soybean oil meal — it may be seen that our results are always on the higher side.

In defatting rice refuse will obviously suffer a loss of lysine which is shown in the amount of 12% by the in vacuo evaporation method and of 15% by the heating evaporation method. Heating evaporation caused a 4% loss of lysine for raw rice refuse and 8% for defatted rice refuse, as compared with in vacuo evaporation. One cause of the apparent loss of lysine in the course of heat evaporation of hydrolysates and defatting of feeds may be sought in the possible racemization of lysine.

The recovery rate lysine added to defatted rice refuse amounted to 97%. It follows that the added lysine is subject to denaturation such as racemization etc. to a very limited extent during hydrolysis. Kofranyi (15) has reported that when the carbohydrate content is ten times that of protein, lysine is almost entirely destroyed during hydrolysis, but as in the case of rice refuse, where the carbohydrate content is only three or four times that of protein, very

little lysine seems to be destroyed.

Our method employing lysine decarboxylase has the advantage of enabling us to determine the quantity of lysine directly from the hydrolysates, and we have little need to consider the loss of lysine in the experimental procedures, so this method is simpler and more accurate than hitherto available methods. By this method, adequate results may be obtained in quantitative analysis of feeds for lysine content, if only proper conditions are adhered to in hydrolysis and other main processes.

Summary

1. By quantitative estimation of lysine in rice refuse, wheat bran, ground wheat, white dent corn, soybean oil meal and fish meal by the use of lysine decarboxylase, we have obtained the values of 7.31, 5.30, 3.00, 2.08, 6.38 and 8.01 % respectively.

2. The lysine content was found to suffer a loss by the heating evaporation of hydrolysates, by heating and defatting of the feeds by ether.

3. The recovery of lysine, added as lysine·HCl to defatted rice refuse and thereafter subjected to hydrolysis, amounted to 97%.

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