XVIII.—*Note on the Nitrogenous Matters in Grass and Ensilage from Grass.*

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That grass and other fodder crops, like most crude vegetable products, contain nitrogenous matters other than albuminoïds, is well known, also that the amount of these matters depends largely on the relative stage of development of the plant, the less mature plants containing a larger percentage of non-albuminoïd nitrogen: but it is still common, in expressing the results of analyses, to represent the whole of the nitrogen as existing in the form of albuminoïds. Our knowledge of the nature of these nitrogenous matters is yet very imperfect. Ammonia and nitric acid have been detected in some cases, and possibly in some cases alkaloïds and peptones, but the principal part of the non-albuminoïd nitrogen of grass appears to exist in the form of amides and amido-acids.

O. Kellner (*Bied. Centr.*, 1879, 270; *Chem. Centr.*, 1879, **10**, 744 and 761; *C. J.*, 1879, Abstr., 819) has determined the amount of non-albuminoïd nitrogen in many specimens of grass and other fodder plants at different stages of growth, the soluble albuminoïds being in most cases precipitated by lead acetate. Of the total nitrogen in the crops from 7·5 to 38·5 per cent. was found to be non-albuminoïd, the higher amounts being, especially in the case of the grasses, in the younger plants.

Peter Collier, in his report to the Commissioner of Agriculture for 1880 (*Rept. of Commis. Agriculture*, 1880, Washington), gives the results of the examination of 15 grasses and three leguminous plants, each at from three to six different stages of growth, and of 37 separate specimens of grasses mostly in full flower. In these analyses the albuminoïds were determined by Stützer's method (*J. f. Landwirthsch.*, 1880) after treatment with water containing a little lactic acid, and precipitation of the dissolved albuminoïds with copper hydrate.

In the great majority of cases the non-albuminoïd nitrogen constituted between 6 and 40 per cent. of the total; in a few cases it was lower; in one case none was present, and in two cases it rose to 50 per cent. of the total nitrogen.

H. P. Armsby (*Rept. Connecticut Expt. Stat.*, 1879) determined the non-albuminoïd nitrogen in 21 samples of fodder, by various processes; its amount was from 8·9 to 39·6 per cent. of the total nitrogen.

The results of other experimenters are in accordance with these. In the examination of several specimens of the principal plants of the hill pastures of Scotland, including not only grasses, but *Cyperaceæ*, *Juncaceæ*, &c., I have found that usually from 10 to 33 per cent. of the nitrogen is non-albuminoïd.

What may be the exact nutritive value of these non-albuminoïd nitrogenous bodies is as yet unknown, but it is certain that the amido-compounds can replace albuminoïds in food to a limited extent only.

Certain amides, as asparagin, can to a certain extent prevent waste of albuminoïds by preventing their oxidation; but cannot entirely supersede them. See Weiske and others (*Zeitschr. f. Biologie*, **15**, 261, and **17**, 415).

Whether, during the fermentations to which grass and other fodder crops are subjected in order to produce ensilage, the albuminoïds which they contain undergo any change into other nitrogenous bodies not having the physiological functions of albuminoïds, and to what extent such changes occur, is a matter not only of much scientific interest, but of great practical importance in considering the economic relations of ensilage. This point has been hitherto unnoticed in published analyses of ensilage, although it seems very likely such changes would occur during the processes, in some respects allied to digestion, to which the fodder is subjected. As a small preliminary contribution, I record analyses of a sample of grass and of the ensilage made therefrom.

The sample of grass was taken from that being filled into a silo, after passing through a chaff-cutting machine, on the farm of Mr. T. R. Hulbert, North Cerney, Gloucestershire, on July 17th, 1883, the silo was filled and closed a few days later.

The grass was coarse, and contained a good deal of miscellaneous herbage, notably thistles and *Ranunculi*. The sample of ensilage was taken from the same part of the silo to which the grass was filled, on December 8th, 1883. The ensilage was not under great pressure, the weight placed on the surface being only about 50 lbs. per square foot. The ensilage when taken out was brown, scarcely acid, and with very slight odour, but after a very short time it began to smell strongly of acetic acid; after some hours, however, this smell disappeared and was replaced by the odour of butyric acid, which was noticeable throughout the homestead in which the ensilage was in use. The ensilage mixed with dry fodder was readily eaten by cattle.

The results of the analyses in percentages are as follows:—

	In fresh substances.		In dry matter.	
	Grass.	Ensilage.	Grass.	Ensilage.
Water, lost at 100° C.	76·93	74·99	—	—
Ether extract	1·27	1·78	5·55	7·12
Total N × 6·25	2·15	2·36	9·32	9·44
Fibre	6·65	7·98	28·82	31·89
Ash	1·96	2·52	8·50	10·10
Soluble carbohydrates, &c., by difference	11·04	10·37	47·81	41·45
	100·00	100·00	100·00	100·00
Albuminoïds by phenol method	1·95	1·06	8·46	4·25
Albuminoïds by copper hydrate method	1·98	1·11	8·57	4·46
Albuminoïds by mercuric hydrate	—	1·14	—	4·55
Albuminoïds by lead hydrate	—	1·18	—	4·72
Per cent. of total nitrogen which is non-albuminoïd, by phenol method	—	—	9·2	54·9

The amount of free acid is not given above; it varied very much with exposure; after exposure for a short time the total amount was found to be equivalent to 0·87 per cent. of acetic acid. The fresh ensilage distilled with water gave only 0·03 per cent. of volatile acid, as acetic acid.

The method referred to as the phenol method is Church's process, consisting in the estimation of the nitrogen, after precipitating all the albuminoïds, with a little metaphosphoric acid and a hot 4 per cent. solution of carbolic acid. In the copper hydrate method, a modification of Ritthausen's, the soluble albuminoïds were precipitated by heating to boiling with recently prepared copper hydrate. In the ensilage the soluble albuminoïds were also precipitated with mercuric chloride and a very slight excess of potassium hydroxide, and with lead acetate and potassium hydroxide, aided by heat. These two methods may be supposed to precipitate any bodies of the nature of peptones, if such were present; they gave slightly higher results than the other methods. The results with the copper hydrate method, with grasses, I have invariably found to be slightly higher than those by the phenol method, possibly owing to the presence of some acid containing nitrogen and forming an insoluble compound with the copper hydrate. All care was taken in collecting and sampling the specimens, and if we assume that they were strictly comparable, though it is difficult to get such specimens with such material, then judging from the amount of ash, there has been a loss of about 18 per cent. of combustible constituents, with a slight loss of water. The

percentage of ether extract has increased, as has always been noticed before in ensilage, sometimes in part owing to the formation of lactic acid. The amount of soluble carbohydrates has decreased through fermentation, and has not been reinforced to the full extent by alteration in the fibre, which consequently has slightly increased in percentage. Such changes are exactly in accord with analyses of clover-hay and ensilage by Dr. A. Voelcker, quoted in the *Field* of January 20, 1883, and of sainfoin and its ensilage by Weiske and of others.

But the most striking change is in the condition of the nitrogenous matters; in the grass only 9 per cent. of the nitrogen is non-albuminoïd, and in the ensilage nearly 55 per cent., reckoned from the albuminoïds by the phenol method. The increase in the percentage of total nitrogen in the ensilage was about one-half only of the increase in percentage of ash, indicating that a loss of nitrogen may have taken place.

The only published analyses bearing on this point are some in which the soluble nitrogenous matter, considered, however, simply as albuminoïds, and the insoluble nitrogenous matter were determined separately. Voelcker, quoted in the *Times*, March 21, 1883, found 57 per cent. of the nitrogenous matter of rye ensilage soluble in water, and 40 per cent. of the nitrogen of maize ensilage.

Sutton (*Field*, February 10, 1883, *Chem. News*, **47**, 287) gives two analyses of hay and two of ensilage made from the same two grasses. In these cases the fermentation had apparently not proceeded to the same length, or, perhaps, in the same direction, as in the ensilage above mentioned, for the percentage of ash was not higher in the ensilage, and the soluble carbohydrates had increased, whilst the indigestible fibre had decreased; but here again the soluble nitrogenous matter had increased from 12 per cent. in the hay to 53 and nearly 60 per cent. in the ensilage. Doubtless the whole of the soluble nitrogenous matter in these cases was not albuminoïd.

Whether such changes in the nitrogenous matter always take place in ensilage, or, as is likely to a less extent when the fodder is subjected to greater pressure, and what exact forms of nitrogenous bodies are produced (only traces of ammonia were found in this case), and whether and to what extent the albuminoïds are lost as food are points requiring investigation; I hope ere long to be able to throw light on some of them.
