

CCLXII. THE EFFECT OF DIFFERENT METHODS OF DRYING ON THE BIOLOGICAL VALUE AND DIGESTIBILITY OF THE PROTEINS AND ON THE CAROTENE CONTENT OF GRASS

BY STEPHEN BARTLETT, KATHLEEN MARY HENRY, STANISŁAW KAZIMIERZ KON, LESLIE WILLIAM OSBORNE, SYDNEY YAN THOMPSON AND JOSEPH TINSLEY

From the National Institute for Research in Dairying, University of Reading

(Received 28 September 1938)

ONE of the outstanding features claimed for modern methods of artificial grass drying is that they involve but little destruction of the nutritive properties of the grass. The high temperatures attained during the process, however, raise the question of heat injury to the proteins. It is well known that heating lowers the biological value of the nitrogen of various foodstuffs [Morgan, 1931; Kon & Markuze, 1931; Fixsen & Jackson, 1932; Maynard & Tunison, 1932; Morgan & Kern, 1934; Morris *et al.* 1936]. Morris *et al.* [1936] found, on the whole, little difference between the biological values for cows of the proteins of fresh and artificially dried grass, though with spring grass there was an indication that the dried product was inferior. In view of the increasing use of artificially dried grass in the feeding of pigs and of poultry and of marked differences between herbivora and omnivora in the utilization of the nitrogen of feeds it was of interest to study on another animal the effect of heat treatment on the proteins of grass. Rats have been extensively used for such tests. They respond readily to even small alterations in the chemical make-up of the nitrogenous constituents of their diets. Further, it is probable that they are less able than ruminants to utilize breakdown products of proteins and are therefore more sensitive in indicating the possible detrimental effects of heating. It is probably safe to assume that if a rat test discloses no difference between two products, a feeding test on cows would likewise show no difference. On the other hand, a definite difference in this respect found with rats would certainly call for a further investigation with ruminants.

For these reasons it was decided to carry out nitrogen balance experiments on rats. The carotene content of the grasses dried under various conditions was also studied, as well as the rate of destruction of carotene in the process of natural curing.

EXPERIMENTAL

(1) *The drying and preparation of grass*

Compared with that of herbivora, the capacity of the intestinal tract of the rat is limited and it was necessary in these experiments to cut down as far as possible the bulk of the diet by selecting a grass of very high protein content. For the same reason (apart from other difficulties) it was not possible to feed the grass fresh and only dry samples could be compared.

Grass was taken from permanent grassland with a first class sward about 4–6 in. in length. The chief plants in the herbage were wild white clover and perennial rye grass. The mower was run over the meadow on 25 June 1937 to remove all long grass and on 21 July the grass was cut with a close cutting mower with an elevator attached so that the freshly cut grass was loaded on a cart immediately and taken to the farm buildings for drying.

The details of the various treatments applied to this batch of grass were as follows.

(a) Grass dried artificially in a grass drier at the normal running temperature (300° F.).

The drier used was a Kaloroil drier of the rotating drum type heated by oil fuel.¹ A full description of this type of drier will be found in the report by Roberts [1937]. The inlet gases were heated to about 300° F. (variation 275–325° F.) but the outlet temperature could not be recorded.

The grass was removed from the drier as soon as it was sufficiently dry (15–30 min.) and it is probable that only the outside of the grass ever reached a temperature approaching 300° F. The freshly dried grass was crisp but after removal from the drier quickly became less powdery. The interval between cutting and drying this sample was 6 hr.—i.e. 2 hr. on the cart and 4 hr. exposure on a wooden platform under a Dutch barn. The condition of this sample after drying was excellent and it was dark green in colour. It was stored in sacks for 15 days and was then made into bales under high pressure and was covered with sacking.

(b) Grass dried artificially in a grass drier at low temperature (170° F.).

The interval between cutting and drying this sample was 6½ hr. It was dried in the same plant as the first sample but the temperature of the inlet gases was kept at about 170° F. (variation 150–200° F.). The time required for drying was much longer and it was necessary to keep the grass in the drum for about an hour (50–70 min.). A well-dried dark green sample of grass was eventually obtained and the after treatment was the same as for the first sample.

(c) Grass dried artificially in a current of hot air.

A third batch of the grass was placed half an hour after cutting in a tunnel-shaped drying oven through which passed a current of air heated by a hot water radiator and propelled by a fan. The temperature of the inlet air was about 135° F. (variation 104–142° F.). The time of drying was 22 hr. This sample was sacked but not pressure-baled.

(d) Grass sun-dried under natural conditions (hay).

A further batch of grass was unloaded from the cart 2 hr. after cutting. It was intended to place the grass in the open at once but as heavy rain fell while unloading it was left overnight indoors spread on a floor. The depth of the layer was about 8 in. and it was turned to avoid undue fermentation. Twenty-one hr. after cutting the grass was spread in the open in a layer about 3 in. thick. The weather to which the grass was subjected was unsettled and dull, and heavy rain fell in the 36th hr. after cutting. The grass was turned on the 3rd day. In order to complete the drying on the 5th day after cutting the grass was turned three times and finally placed in a small stack and thatched with straw. The hay had lost most of its green colour and parts were slightly blackened but would be described by a farmer as moderate quality hay. Ten days later this hay was pressure-baled and the bales were covered with sacking for storage.

In addition to these samples, a further batch of grass was cut from the same meadow one day later and was treated as follows.

¹ We are indebted to Messrs Kaloroil Burners, Ltd., for the loan of the drier.

(e) Grass dried in air at normal temperature without sun or rain.

The grass was cut, carted and off-loaded after an hour. It was spread about 3 in. deep on a concrete floor in a room with screened windows. The grass was turned twice daily to prevent fermentation and was thoroughly air-dried in 12 days. It was pressure-baled 14 days after cutting and the bales were covered up with sacking. The hay lost much of its green colour during curing but otherwise appeared to be of very good quality.

Finally, to study the rate of destruction of carotene in the course of curing, part of a different meadow was scythed between 11 and 11.30 a.m. at the beginning of September, the grass was left in the field in a layer of about 3 in. and samples were withdrawn at intervals for carotene estimations. This field had been previously cut at the beginning of June. Between then and the second cutting the weather was, on the whole, very dry, and the grass made little growth; it was 6–7 in. high when cut and rather parched. During curing the weather was, on the whole, bright and sunny with no rain.

(2) *Determination of the biological value and digestibility of the proteins of dried grass*

It was technically not possible to compare simultaneously more than three types of dried grass and the tests were therefore limited to three products of practical importance: two samples of grass dried in the drier at high and low temperature and the sample of hay. The feeding experiments started 5½ months after the grass had been dried.

The grasses dried at high and low temperature contained respectively 23.5% and 23.1% of protein ($N \times 6.25$) on the dry basis. The sun-cured sample contained more, 25.1%, probably owing to relatively greater loss of fermentable non-nitrogenous constituents.

The grasses were milled to a fine powder and were mixed in suitable proportions with a "nitrogen-free" basal diet to yield mixtures containing about 9% protein ($N \times 6.25$) on the dry basis. The "nitrogen-free" diet had the following composition:

Butter fat	17
Rice starch	51
Potato starch	10
Sugar	17
Salts (Steenbock 40)	5

The biological value and true digestibility of the grasses were measured by the method of Mitchell [Mitchell, 1924; Mitchell & Carman, 1926] as described in detail by Henry *et al.* [1937]. Because of the nature of the grass diets, it was necessary to mix them for feeding with a relatively large quantity of water (more than 1½ times the weight of the diet). Twelve rats were used and on each the three types of grass were tested in turn. The results are given in Tables I, II and III. Table I gives the individual nitrogen metabolism data, Table II gives average values and Table III contains a statistical examination of differences in biological values. Figures for true digestibilities were so similar (Table II) that statistical tests were considered superfluous. The statistical method used was that of the paired *t* test of "Student" [1908; 1925] as described by White [1937]. When comparing two substances results obtained on the same rat in different periods were paired. The results show quite conclusively that sun-drying was more detrimental than artificial drying at either high or low temperature. The figures also indicate that the proteins of grass dried at low temperature were inferior to those of the high-temperature grass but the evidence to this

Table I. Nitrogen metabolism; individual data for 6-day periods

Rat no.	Type of grass*	Initial wt. g.	Final wt. g.	Diet intake† g.	N intake mg.	Faecal N mg.	N in urine mg.	Biological value	True digestibility
Low egg-nitrogen diet									
1	—	64	70	46.11	—	101.5	100.0	—	—
2	—	65	69	46.94	—	89.8	102.2	—	—
3	—	69	73	44.91	—	92.9	110.9	—	—
4	—	59	64	37.67	—	93.6	98.2	—	—
5	—	58	60	31.82	—	73.5	111.1	—	—
6	—	60	68	49.59	—	105.0	113.8	—	—
7	—	64	71	43.73	—	103.8	106.8	—	—
8	—	62	66	37.38	—	75.6	101.2	—	—
9	—	64	73	47.45	—	97.8	106.2	—	—
10	—	60	63	40.61	—	99.4	108.3	—	—
11	—	59	62	35.14	—	90.4	98.4	—	—
12	—	61	62	38.86	—	98.0	106.3	—	—
1st grass period									
1	S	68	69	40.31	566.4	265.7	297.5	51.7	69.9
2	H	71	72	38.59	544.9	275.1	257.9	57.3	64.5
3	L	72	75	44.54	624.5	311.8	274.6	59.7	64.9
4	S	61	59	34.93	490.8	257.9	282.2	39.5	64.3
5	H	64	66	40.32	569.3	302.6	241.1	66.0	63.9
6	L	64	64	35.54	498.3	261.6	264.9	50.2	63.1
7	S	71	67	36.70	515.6	264.8	269.8	50.8	65.7
8	H	70	72	42.78	604.1	308.6	273.8	57.2	63.5
9	L	71	73	41.17	577.2	282.8	288.3	53.1	66.5
10	S	63	63	42.04	590.7	297.0	328.1	43.5	67.1
11	H	62	68	47.51	670.8	331.0	261.5	65.9	69.2
12	L	62	66	44.62	625.6	312.6	294.6	55.8	68.5
2nd grass period									
1	H	77	79	45.39	640.9	373.2	225.7	73.4	59.6
2	L	77	82	48.14	674.9	364.2	255.3	67.4	62.5
3	S	79	80	47.49	667.2	376.1	275.1	59.1	58.5
4	H	64	69	42.34	597.8	299.3	228.0	66.9	65.7
5	L	70	73	45.07	631.9	322.5	246.3	71.0	66.9
6	S	68	70	43.75	614.7	336.9	284.1	54.0	61.3
7	H	72	83	55.70	786.5	381.6	262.1	72.0	68.6
8	L	73	77	46.52	652.2	348.4	272.5	60.3	61.6
9	S	75	74	44.41	623.9	333.6	316.5	47.4	62.8
10	H	68	71	45.58	643.6	261.5	245.8	72.3	76.5
11	L	71	73	45.86	643.0	344.2	239.9	69.7	66.1
12	S	66	68	44.30	622.4	333.7	299.8	52.0	65.3
3rd grass period									
1	L	78	81	53.34	747.8	411.7	347.4	54.8	64.0
2	S	79	83	51.56	724.4	370.4	319.7	58.1	66.8
3	H	84	94	57.96	818.4	444.6	257.0	73.0	60.6
4	L	71	78	49.42	692.9	339.5	263.3	65.4	66.0
5	S	76	78	49.93	701.5	355.4	314.6	60.4	68.0
6	H	73	83	57.23	808.1	430.7	269.0	70.6	63.2
7	L	85	91	56.00	785.1	423.8	276.6	68.2	63.5
8	S	75	81	53.82	756.2	404.2	331.3	53.7	61.8
9	H	75	85	57.14	806.8	426.5	294.5	65.3	64.1
10	L	76	84	56.31	789.5	382.1	278.8	70.3	68.8
11	S	74	76	50.86	714.6	418.9	309.0	52.1	61.6
12	H	71	79	52.10	735.7	366.5	281.7	66.7	69.5
Low egg-nitrogen diet									
1	—	80	84	38.57	—	108.9	138.6	—	—
2	—	85	86	34.18	—	92.8	122.0	—	—
3	—	96	101	48.98	—	103.9	130.8	—	—
4	—	83	95	55.09	—	108.6	119.6	—	—
5	—	78	80	34.53	—	94.9	122.6	—	—
6	—	87	96	57.36	—	137.7	132.4	—	—
7	—	95	105	59.13	—	146.6	126.6	—	—
8	—	85	91	41.92	—	91.6	126.4	—	—
9	—	92	93	43.32	—	108.5	130.7	—	—
10	—	87	95	52.81	—	126.6	125.0	—	—
11	—	77	81	40.19	—	118.0	116.2	—	—
12	—	85	85	38.41	—	107.2	120.0	—	—

* S=sun-dried. H=artificially dried at high temperature. L=artificially dried at low temperature.

† On the dry basis.

Table II. *Biological values and true digestibilities of three types of dried grass*

Period	Sun-dried		Artificially dried			
	Biological value	True digestibility	High temperature		Low temperature	
			Biological value	True digestibility	Biological value	True digestibility
I	46.38	66.75	61.60	65.28	54.70	65.75
II	53.11	61.97	71.15	67.60	67.10	64.28
III	56.08	64.55	68.90	64.35	64.68	65.57
Mean	51.86	64.42	67.22	65.74	62.16	65.20

Table III. *Statistical significance of the mean differences in the biological values of the three types of dried grass*

Difference	Standard error of mean	P*
High temperature - Sun-dried	+ 15.36 ± 2.63	1 : 5000 Significant
Low temperature - Sun-dried	+ 10.30 ± 2.81	1 : 263 Significant
High temperature - Low temperature	+ 5.06 ± 2.85	1 : 10 Not significant

* P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

effect is inconclusive (Table III). The lowering of the biological value of the proteins in the sun-curing of grass is most probably associated with the fermentative changes taking place during the process. It is sufficiently marked, as judged on rats, to invite an investigation on ruminants. According to Saakian [1935] artificially dried hay was 20% higher in feeding value than ordinary hay from the same meadows.

Nevens [1921] has measured on rats the biological value and true digestibility of the proteins of alfalfa (lucerne) hay and obtained values of 62 and 58 respectively. The former figure is definitely higher than that obtained in the present work for sun-cured grass, slightly lower than that for grass dried at high temperature and equal to that for the low-temperature grass. The digestibility coefficient of the lucerne hay was lower than that of the artificially dried grasses which amounted to about 65.

(3) *The effect of drying on the carotene content of the various grasses*

The carotene was estimated by the method of Ferguson & Bishop [1936]. The results will be found in Tables IV and V of which the first gives the carotene

Table IV. *The carotene content of grass dried by various methods*

Method of drying	% moisture	Mg. carotene/100 g. grass		Carotene content as % of that present in fresh grass
		Moist	Dry	
Fresh grass	88.92	10.4	49.3	100
		10.2	48.5	
Artificially dried high temperature	10.56	39.1	42.4	86.7
Artificially dried low temperature	12.90	32.1	38.2	78.1
Artificially dried in current of hot air	7.89	39.0	43.7	89.4
Sun-dried under natural conditions (hay)	21.38	9.6	12.2	25.0
Dried without sun at air temperature	18.06	12.6	15.4	31.5

Table V. *Rate of destruction of carotene in the sun-curing of grass*

Hours after cutting	Time of exposure to sunshine* hr. min.	% Moisture	Mg. carotene/100 g. grass		Carotene content as % of that present in fresh grass
			Moist	Dry	
Freshly cut	—	65.27	8.7	25.0	100
1	54	59.05	9.0	22.0	88
3	2 54	47.78	11.0	21.0	84
6	5 27	38.59	10.3	16.8	67.2
24	9 57	35.54	9.9	15.4	61.6
48	16 32	35.09	9.1	13.4	53.6
72	24 32	24.78	4.7	6.3	25.2
96	30 45	16.17	4.4	5.3	21.2

* As measured by a Jordan photographic sunshine recorder.

content of the grasses used in the protein experiment and the second shows the changes in the carotene content of grass in the course of its conversion into hay.

In both cases hay cured under natural conditions contained only about one quarter of the carotene originally present in the fresh grass. Artificial drying at normal running temperature preserved nearly 90% of the carotene. This figure is in good agreement with the earlier findings of Watson *et al.* [1933]. The drying at the lower temperature was slightly more injurious, most probably because of the longer exposure of the material to the hot gases after it had become relatively dry [Watson, 1934] and it is possible that the same reason would account for the slight lowering of the biological value of the proteins described on p. 2028.

The high figure for the carotene content of the sample of grass dried in a hot air tunnel is rather surprising as the drying took some 24 hr. and air was continuously passing over the grass; on the other hand the temperature of the air never rose above 142° F. and that of the grass must have been even lower for, at any rate, part of the time.

The measurements of the rate of disappearance of carotene when grass is made into hay show a fairly steady fall. The loss after 48 hr. is some 46% of the original value. This compares unfavourably with the figures given by Greenhill [1936], but it must be remembered that his results were obtained in the course of partial field drying of grass as a preliminary to artificial drying and not in haymaking. On the other hand, the figures agree quite well with the data of Dexter & Moore [1937] for lucerne hay cured in swaths.

SUMMARY

1. Grass was dried by artificial means and also made into hay in the normal way and the effects of the various procedures on the carotene content and on the biological value of the proteins of grass were measured.

2. The biological value and true digestibility of the proteins of grass artificially dried at the normal running temperature (300° F.) in a rotating drum type drier were compared on rats by the method of Mitchell with those of grass dried in the same drier at low temperature (170° F.) and those of sun-cured hay. All three samples were made from one bulk of fresh grass.

3. The following figures were found for biological value and true digestibility: artificially dried grass, high temperature, 67 and 66; artificially dried grass, low temperature, 62 and 65; hay, 52 and 64.

The differences in biological values of the proteins between artificially dried samples and hay were statistically highly significant. Other differences were not.

4. Carotene figures are given for various samples of dried and fresh grass and also measurements of the rate of loss of carotene in the course of sun-curing of grass.

Our thanks are due to Prof. H. D. Kay and Mr J. Mackintosh for advice during the course of these experiments; also to Mr J. E. Campion who assisted in operating the grass-drying plant.

Note added 28 October 1938. Smuts & Malan [1938] have obtained very recently for lucerne meal biological values of 60 and 61 which agree very well with the findings of Nevens [1921].

REFERENCES

- Dexter & Moore (1937). *Quart. Bull. Mich. agric. Exp. Sta.* **20**, 75.
 Ferguson & Bishop (1936). *Analyst*, **61**, 515.
 Fixsen & Jackson (1932). *Biochem. J.* **26**, 1923.
 Greenhill (1936). *Emp. J. Exp. Agric.* **4**, 145.
 Henry, Kon & Watson (1937). Milk and Nutrition, Part 1, p. 37. Reading: Nat. Inst. Res. Dairying.
 Kon & Markuze (1931). *Biochem. J.* **25**, 1476.
 Maynard & Tunison (1932). *Industr. Engng Chem.* **24**, 1168.
 Mitchell (1924). *J. biol. Chem.* **58**, 873.
 — & Carman (1926). *J. biol. Chem.* **68**, 183.
 Morgan (1931). *J. biol. Chem.* **90**, 771.
 — & Kern (1934). *J. Nutrit.* **7**, 367.
 Morris, Wright & Fowler (1936). *J. Dairy Res.* **7**, 97.
 Nevens (1921). *J. Dairy Sci.* **4**, 552.
 Roberts (1937). *Rep. Ser. agric. Res. Coun., Lond.*, **2**.
 Saakian (1935). *Probl. Zivot.* No. 8, 55.
 Smuts & Malan (1938). *Onderstepoort J. vet. Sci.* **10**, 207.
 "Student" (1908). *Biometrika*, **6**, 1.
 — (1925). *Metron*, **5**, 105.
 Watson (1934). *J.R. agric. Soc.* **95**, 103.
 — Drummond, Heilbron & Morton (1933). *Emp. J. exp. Agric.* **1**, 68.
 White (1937). Milk and Nutrition, Part 1, p. 64. Reading: Nat. Inst. Res. Dairying.