Part III

Report on experiments on variation in nutrient composition and digestibility of DDGS (Distiller's Dry Grains with Solubles)

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I. Purpose

The parties involved in trading feed grains and other feed materials are normally aware of the relationships of the difference of their external appearance with their nutrient composition and usefulness. Several reports are available on nutrient composition of DDGS and its nutritional evaluation in different animal species. Some manufacturers and the University of Minnesota have published data on variation in the nutrient composition of DDGS. There are, however, very few reports on the relationships between the external appearance of DDGS and its nutrient composition, digestibility, etc. Indices for evaluating the nutritional value of DDGS at the time of its purchase have not been established.

We therefore analyzed the nutrient composition and digestibility of different DDGS samples and examined their variation. Among the properties such as color, smell, shape, etc that humans can perceive, we selected color, which is relatively easy to assign objective values to, and studied the relationships of the color of DDGS with its nutrient composition and digestibility.

II. Materials and Methods

1. Materials

A total of 22 DDGS samples were examined. Of these, 20 were of DDGS prepared by the new method. They had been obtained from USA by the Tokyo Office of the US Grains Council, who had also got the usually determined five components and the energy content analyzed. The two other samples were also obtained from the same Tokyo Office of the Council. But they were of a darker color and it is believed that they had been prepared by the older method. Table 1 gives the contents of the five components and the total energy content of the 22 samples.

Table 1 Composition and total energy content of the DDGS samples studied

Sample No.	Water	Crude protein	Crude fat	Soluble Nitrogen-free extract	Crude fiber	Crude ash	Total energy
1	9.4	28.1	12.0	39.5	7.1	4.0	5.00
2	12.3	25.9	12.3	36.1	7.0	5.0	4.91
3	11.0	30.0	13.1	35.7	6.6	4.1	4.92
4	13.1	26.5	13.0	36.7	6.8	4.1	4.93
5	9.9	25.8	11.7	37.2	6.5	3.6	4.98
6	11.4	25.9	10.7	43.1	6.6	3.9	4.86
7	10.0	27.8	11.8	36.9	7.5	4.6	4.88
8	12.1	27.3	12.4	37.9	7.3	4.1	4.90
9	9.1	28.6	10.5	41.2	6.1	4.4	4.76
10	12.3	26.0	12.4	39.2	6.8	4.3	4.92
11	11.1	29.5	11.7	36.0	6.8	3.7	4.90
12	12.8	26.4	11.8	38.5	8.4	3.5	5.01
13	8.4	29.0	12.7	38.2	6.5	4.4	5.00
14	13.1	26.7	12.0	39.8	6.8	3.7	4.99
15	9.8	28.2	12.5	36.9	6.7	4.1	4.90
16	14.0	23.8	13.5	39.0	6.0	4.0	4.98
17	9.7	28.5	11.9	38.7	8.0	3.4	4.99
18	12.3	25.5	12.4	38.6	6.8	4.0	4.90
19	12.7	29.1	12.3	38.8	6.9	4.2	4.85
20	13.1	27.4	11.6	37.2	6.7	3.9	4.96
21	10.5	29.8	9.9	39.1	6.5	4.2	4.51
22	10.4	22.3	2.9	51.3	7.9	5.2	4.23
Mean of samples 1-20	11.4	27.3	12.1	38.3	6.9	4.1	4.93
S.D.	1.6	1.6	0.7	1.8	0.6	0.4	0.06
Coeff. of variation (%)	14	6	6	5	8	9	1
Mean of samples 21 & 22	10.5	26.1	6.4	45.2	7.2	4.7	4.37

(Units: %, Mcal/kg)

1- 20: Analyzed at Japan Scientific Feeds Association
21, 22: Analyzed at the Laboratory of Animal Nutrition, Nippon Veterinary and Animal Science University

2. Study period

October 2003 to April 2004

3. Study location

Laboratory of Animal Nutrition, Nippon Veterinary and Animal Science University (Tokyo)

4. Observations

(1) Apparent color depth of DDGS

Five panelists ranked the color depth of DDGS, as seen by the eye, from 1 (brightest) to 22 (darkest). This rank score was designated as "apparent color depth".

(2) Color measurements with a color difference meter

The luminosity (L), redness (a) and yellowness (b) of each sample were measured with a color difference meter.

(3) Water-soluble dry matter and water-soluble nitrogen

The dry matter and nitrogen content in the water-soluble fraction were determined, after soaking the samples in 20ºC(68ºF) water for 12h, as indices of digestibility of dry matter and nitrogen.

(4) Fermentation pattern in an artificial rumen model

In a fermentation study of DDGS in an artificial bovine rumen (a laboratory-scale digestion test using the rumen fluid of dairy cattle in which the sample was decomposed by the ruminal microbes), gas production by the decomposition of DDGS was measured at different time points as an index of its digestibility by cattle.

(5) Dry matter digestibility in an artificial rumen

The digestibility of dry matter (in vitro dry matter digestibility or IVDMD) was measured after 10h in the above-described fermentation test in an artificial rumen.

5. Analysis of correlations

Correlations among the 5 parameters listed above were examined and the relationships between the color of the DDGS and the analyzed nutrient contents and digestibility were examined through linear regression.

III. Results and discussion

1. Six commonly analyzed components and total energy

Table 1 gives the mean values of the 6 commonly analyzed components, the measured values of the total energy content, and their standard deviations and coefficients of variation for the 22 samples used in the study. The 20 different samples (manufacturers) of DDGS produced by the new method had relatively small variation in composition, with coefficients of variation of not more than 10%, except for water for which it was 14%. In ordinary feed grains and by-products, the coefficient of variation is mostly higher than 10%. Thus, DDGS may be said to have a comparatively small variation in composition.

Samples 21 and 22 were dark in appearance. Their crude fat content was low compared to the other 20 samples and the total energy was also correspondingly low. These analyzed values reflect the characteristics of DDGS produced by the new method and the old method.

2. Relationship between apparent color depth and measured color values of DDGS

The luminosity (L), redness (a) and yellowness (b) of the DDGS samples measured by the color difference meter are given in Table 2. The L-value was 54, a-value was 9 and b-value about 24, which represent a shade with a moderate luminosity and a slightly reddish and strongly yellow shade, as perceived by the eye. Among these values, b showed the largest variability, with a coefficient of variation of 15.8%. The DDGS samples produced by new method had a color difference that was discernible by the eye when samples placed side by side were compared, but difficult to detect by the ordinary method of observing single samples.

Samples 21 and 22 had a clearly darker color and could be easily differentiated by visual observation from the DDGS samples produced by the new method. Their L-value was 35 and b-value 14, which were very different from the values of the other samples.

There was a high correlation between the apparent color depth of DDGS and the values measured by the color meter. These relationships are shown in Table 3. The results suggested that the panelists perceived the strongly reddish and weakly yellowish DDGS samples as being darker in color. The apparent color depth was particularly well-correlated with the L-value (correlation coefficient: -0.86, $P<0.001$). Therefore, the L-values can be used in place of apparent color depth in statistical analysis of the values.

Table 2 Color values of DDGS measured by color difference meter

	L (Luminosity)	a (Redness)	b (Yellowness)
Mean of samples 1-20 (Coeff. of variation, %)	53.78±4.10 (7.6)	8.68±1.14 (13.1)	24.24±3.84 (15.8)
Mean of samples 21 and 22	35.01	9.10	14.20

Table 3 Coefficients of correlation between apparent color depth of DDGS samples and their color values measured by the color difference meter

<table>
<tr><th colspan="2"></th><th>Apparent color depth</th><th>L (Luminosity)</th><th>a (Redness)</th><th>b (Yellowness)</th></tr>
<tr><td rowspan="2">Measured luminosity (L)</td><td>r (Correlation coeff.)</td><td>−0.86</td><td>—</td><td>−0.51</td><td>0.82</td></tr>
<tr><td>P (Significance level of correlation)</td><td>***</td><td>—</td><td>**</td><td>***</td></tr>
<tr><td rowspan="2">Apparent color depth</td><td>r (Correlation coeff.)</td><td>—</td><td>−0.86</td><td>0.75</td><td>−0.78</td></tr>
<tr><td>P (Significance level of correlation)</td><td>—</td><td>***</td><td>***</td><td>***</td></tr>
</table>

(***: P<0.001, **: P<0.01)

Fig 1 shows the relationship between the apparent color depth and the value of L. It is clear that the ranking of color depth on the basis of visual observation and the L-value measured with the instrument had a high correlation. The two data points at the right bottom corner are those of the two samples that looked different even to the naked eye.

Fig. 1　Relationship between apparent color depth and the value of L measured with the color difference meter

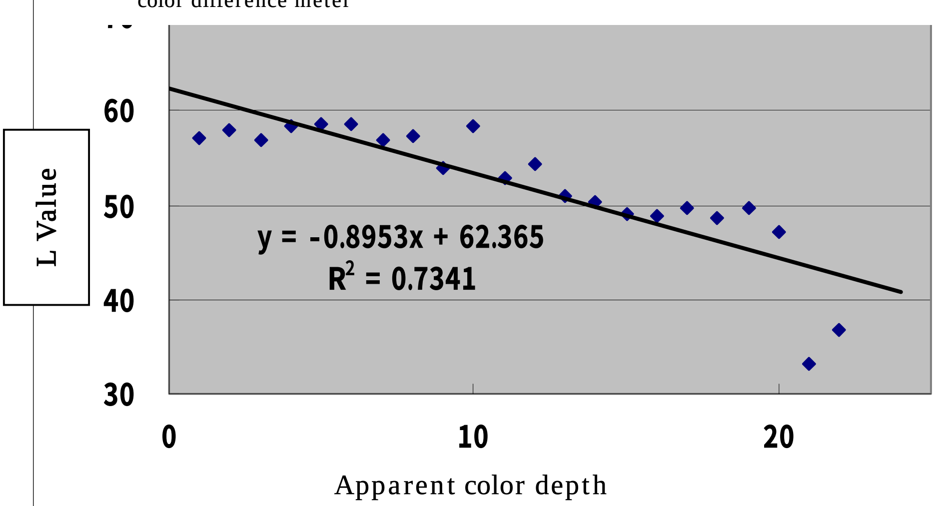


Plate 1 shows the external appearance, along with the apparent color depth rank, of some samples examined in this study.

Plate 1　External appearance of some samples and their apparent color depth

Sample 9 (Apparent color depth=1)　　Sample 12 (Apparent color depth=13)

Sample 910(Apparent color depth=18)

Sample 22 (Apparent color depth=22)

3. Contents of water-soluble matter and water-soluble nitrogen

Table 4 gives the contents of water-soluble matter and water-soluble nitrogen. In general, the water-soluble matter gets digested very well in the digestive tract of animals. So, the content of water-soluble matter can be taken as an index of the content of digestible components in a DDGS sample. The content of water-soluble matter in DDGS was 23.4%, with the rather high coefficient of variation of 13.0%. Water-soluble nitrogen content was 388.9mg/100g, which represented 8.9% of the total nitrogen. It was highly variable, the coefficient of variation being 50%.

The mean content of water-soluble matter in the two samples believed to have been prepared by the old method was 33.1%, considerably higher than in the samples prepared by the new method. Water-soluble nitrogen in these two samples was more than 3 times that in the samples prepared by the new method, being 1287.3mg/100g, and the proportion of water-soluble nitrogen in total nitrogen was 30.1% on the average. Thus, the DDGS samples prepared by the new and those prepared old method differed considerably in water-soluble matter content and this appears to be a characteristic of DDGS prepared by different methods.

Table 4　Contents of water-soluble matter and water-soluble nitrogen*

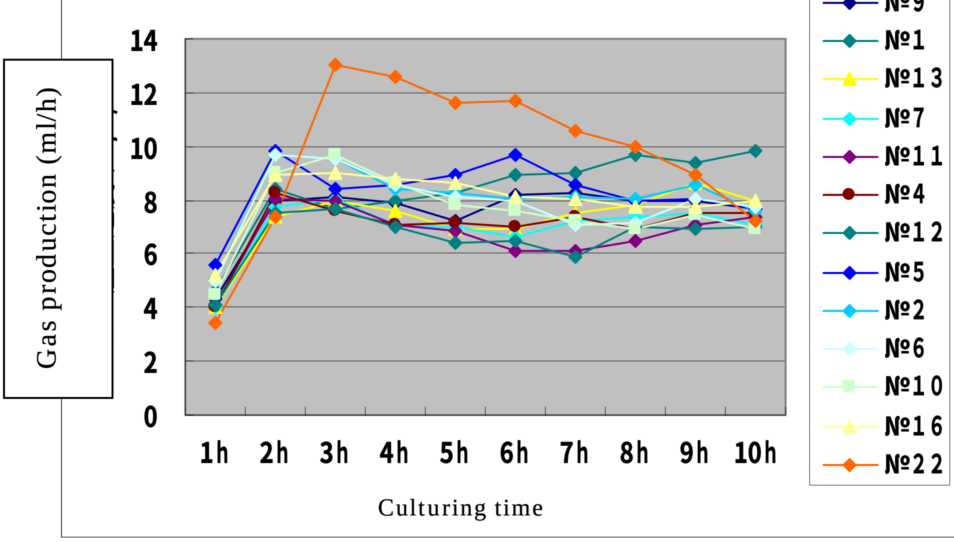
	Water-soluble matter (%)	Water-soluble nitrogen (mg/100g)
Mean of samples 1-20 ± S.D. (Coeff. of variation, %)	23.4±3.0 (13.0)	388.9± 196.1 (50.0)
Mean of samples 21 & 22	33.1	1287.3

(*Both are given on dry matter basis)

4. Pattern of fermentation in the artificial rumen

Change with time in the amount of gas produced in the artificial rumen is shown in Fig 2 as a gas production curves. Theses curves show the pattern of fermentation of nutrient components in the sample, i.e., the time course of the extent of digestion of the nutrients by the ruminal microbes. Therefore, it reflects the digestion characteristics, like the amount of sample digested and the rate of digestion. The DDGS sampled generally had curves with gentle slopes, suggesting that they were digested gradually over a long period of time. There was no major difference among the DDGS samples in the pattern of fermentation. Sample 22, possibly prepared by the old method, showed a unique fermentation pattern with generally high gas production that peaked 3h after the start.

Fig 2.　Time curves of gas production in an artificial rumen model



5. IVDMD in the artificial rumen model

Table 5 shows IVDMD after 10h of culturing in the artificial bovine rumen. The IVDMD of DDGS was 25.5% with 18% variation. Samples believed to have been prepared by the old method had an IDVMD of 38.2%, considerably higher than of samples prepared by the new method. This difference is believed to arise from the difference in the content of water-soluble matter. The results suggest that the digestion of DDGS samples produced by the new method occurred more in the lower digestive tracts, such as the small intestines, than in the rumen because they had higher fat content and lower content of soluble matter.

Table 5 In vitro dry matter digestibility (IVDMD)

Sample	IVDMD（%）
1	23.1
2	29.8
4	24.8
5	30.3
6	27.4
7	20.5
9	23.1
10	25.7
11	22.1
12	25.1
13	25.0
16	28.7
21	38.1
22	38.3
Mean of samples 1-20 ± SD (Coeff. of variation)	25.5±4.6 （18.0）
Mean of samples 21 & 22	38.2

(In 10h in an artificial rumen)

As described above, the IVDMD in the artificial rumen model differed between samples produced by the new method and old method. This difference appears to reflect the characteristics of DDGS prepared by different methods.

IV. Correlation of sample color with nutrient composition and digestibility

1. Correlation between appearance (color) and nutrient composition

The coefficients of correlation between the appearance, the color in particular, and the nutrient composition were calculated. The statistically significant coefficients involving at least one of the parameters, i.e., L-value measured with the color difference meter and apparent color depth, are listed in Table 6. Samples with high luminosity (L-value) had high fat content and high total energy whereas they were low in water-soluble matter and water-soluble nitrogen. Samples with high apparent color depth (dark in color) had low total energy and high content of water-soluble matter.

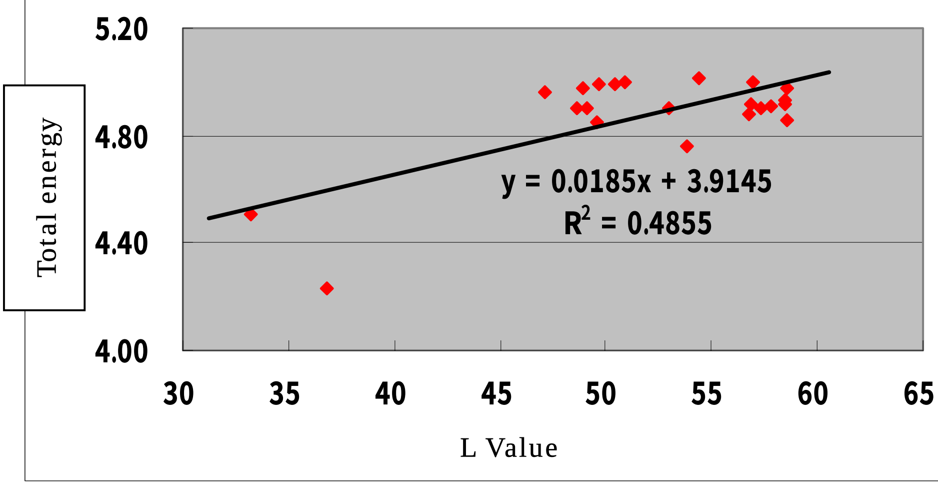
Table 6 Coefficients of correlation of luminosity and apparent color depth with nutrient contents of DDGS samples

		Crude fat	Total energy	Water-soluble matter	Water-soluble N
Measured luminosity (L)	r (Correlation coeff.)	0.58	0.70	-0.55	-0.54
	P (Significance level)	**	***	**	**
Apparent color depth	r (Correlation coeff.)	-0.34	-0.41	0.79	0.36
	P (Significance level)	NS	*	***	NS

(***: $P<0.001$, **: $P<0.01$, *: $P<0.05$, NS: Not significant)

Figs 3 and 4 respectively show the correlation between luminosity (L) and total energy, and L and water-soluble matter. Except for the two samples with low L-values (the two dots at left of these Figs) the samples (the 20 samples prepared by the new method) showed no clear relationship between their luminosity and total energy or water-soluble matter. It appeared that except for the samples with the very dark color there was not much difference in nutrient composition among the samples. The same can be said about the relationship between L and the crude fat content. Other components like protein, starch, fiber, etc did not have significant correlation with sample color.

Fig 3 Luminosity (L) and total energy



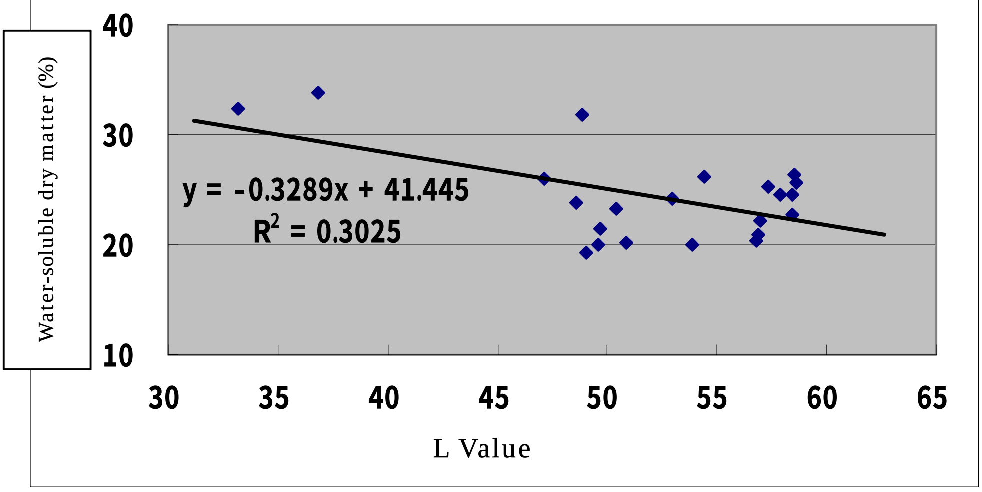


Fig 4 Luminosity (L) and water-soluble matter

2. Correlation between appearance (color) and digestibility

Table 7 gives correlation coefficients of the sample color with digestibility of samples in the artificial bovine rumen. Samples with a higher L-value generally produced a lower amount of gas and the total amount of gas produced in 10h was significantly low in such samples ($P<0.05$). Besides, the IVDMD in 10h, which represents the digestibility in bovine rumen, was also significantly lower ($P<0.01$). With regard to apparent color depth, samples with a dark color had a significantly higher ($P<0.05$) amount of gas production and IVDMD in the early stage of digestion. The IVDMD was the only parameter that was significantly correlated with both the measured luminosity and the apparent color depth; the darker the sample, the higher was its digestibility.

Table 7 Coefficients of correlation between the luminosity and apparent color depth of DDGS samples and their digestibility

		Gas production in the first 4h	Gas production in the last 4h	Total gas production in 10h	IVDMD
Measured luminosity (L)	r (Correlation coeff.)	-0.12	-0.54	-0.40	-0.72
	P (Significance level)	NS	*	NS	**
Apparent color depth	r (Correlation coeff.)	0.61	0.10	0.43	0.66

	P (Significance level)	*	NS	NS	*

(*: P<0.01, **: P<0.05, NS: Not significant)

Of the 20 samples prepared by the new method, the 4 samples with lowest apparent color depth were clearly lighter to the naked eye than the 4 with the highest apparent color depth. So, gas production with time by these 8 samples was determined in the artificial rumen and gas production curves were prepared from the mean values. These are shown in Fig 5. The darker samples clearly showed higher gas production in the early stage of the fermentation, particularly at 2, 3 and 4 h from the start (P<0.001). This result suggests that even among samples produced by the new method, those that have a discernable difference in apparent color depth have a different pattern of fermentation in the rumen of bovines.

Fig 5 Gas production curves of DDGS samples with different apparent color depth, in an artificial rumen.

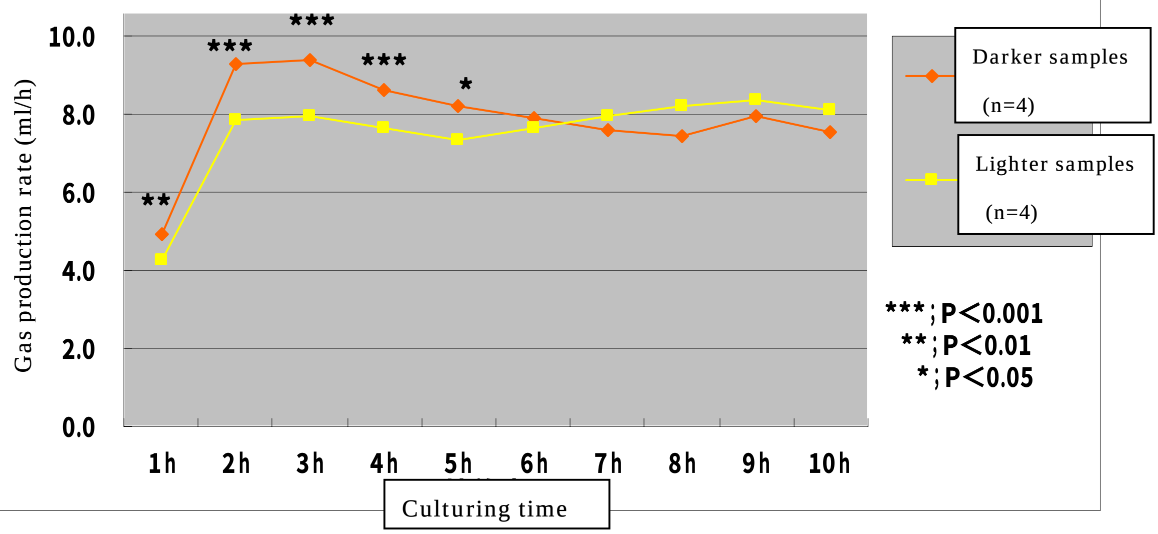
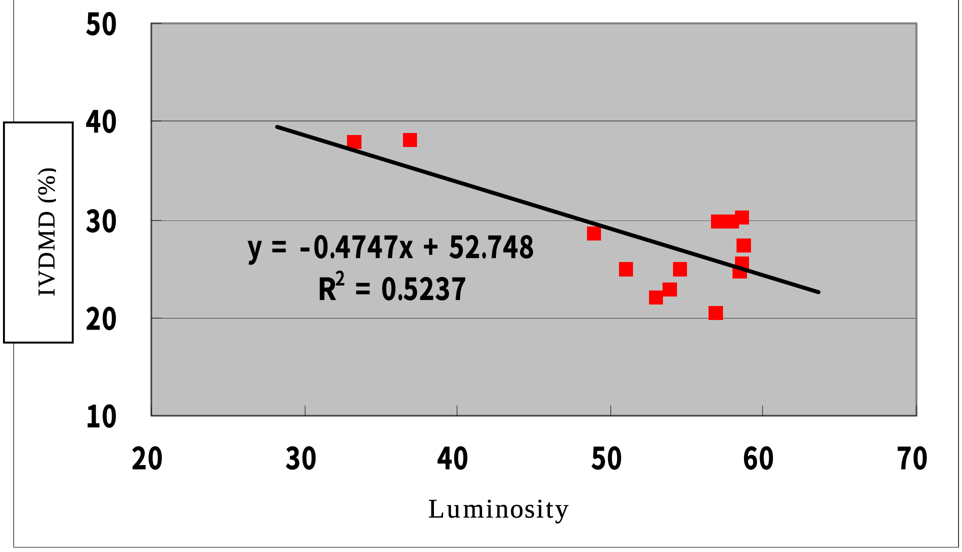


Fig 6 shows the correlation between dry matter digestibility and the measured luminosity (L) when the samples were cultured in the artificial rumen for 10h. The L value was clearly correlated IVDMD (P<0.01). However, except for the samples 21 and 22 (the two data points on the left side of the Fig), which were clearly darker, the samples (20 samples made by the new method) showed low correlation between luminosity and IVDMD, as can be seen from the Fig. Thus, unless the color was very dark, DDGS would not have much variability in dry matter digestibility.

Fig 6 Measured luminosity (L) and dry matter digestibility (IVDMD)



V. Conclusion

1. The study was carried out by the Laboratory of Animal Nutrition, Nippon Veterinary and Animal Science University (Tokyo).
2. The commonly analyzed 6 components, total energy, water-soluble matter and water soluble protein were analyzed in 20 DDGS samples produced by the new method. Their color values were also measured with a color difference meter and the apparent color depth as perceived by the naked eye also evaluated. The pattern of digestion and the in vitro dry matter digestibility (IVDMD) of the DDGS samples was evaluated, as indices of the digestibility of DDGS feed, by a fermentation test in an artificial rumen model containing bovine ruminal fluid.
3. The variation in their nutrient composition and the digestibility indices were analyzed and compared with those of two other samples that were clearly darker in color and are believed to have been prepared by an older method.
4. The correlation between color and nutrient composition and between the color and digestibility indices was studied to enable the estimation of nutritional value of DDGS from its external appearance.
5. The commonly analyzed 6 components of DDGS samples prepared by the new method, which were lighter in color, had coefficients of variation of not more than 10% except for the water content, and therefore, can be said to have small variability.
6. The content of water-soluble matter was about 23% and its variability was large, with a coefficient of variation about 15%. Water-soluble nitrogen was about 9% of the total nitrogen content. This component showed even higher variability, the coefficient of variation being 50%.
7. In the fermentation tests conducted with the artificial rumen, the DDGS samples did not differ significantly in their pattern of fermentation, all of them showing gas production curves with gentle slopes. This result suggests that DDGS is a kind of feed that is digested in a steady manner over a long period of time.
8. Samples with high luminosity (L) measured by the color difference meter had a high fat content and high total energy, and low contents of water-soluble matter and water-soluble nitrogen. DDGS with high apparent color depth (dark in color) had low total energy and high water-soluble matter content. The 20 DDGS samples produced by the new method had high measured luminosity and relatively low variation in apparent color depth evaluated by the eye. Their color had low correlation with the contents of these nutrients and with digestibility.
9. The two DDGS samples that were particularly dark in color had a high content of

nitrogen-free extract but were low in crude fat content because of which they were low in total energy content. They were high in the content of water-soluble matter, particularly water-soluble nitrogen. In the artificial rumen test, these two samples showed a unique fermentation pattern with a generally high gas production that peaked 3h after the start.

10. From the above results, we can conclude that the DDGS prepared by the new method has low variability in their components compared to ordinary grains and byproducts and is a feed material with uniform quality.