Correlation of Furosine Content in Dried Distillers Grains with Solubles with Rumen Degradation Characteristics and Small Intestinal Digestibility of Rumen Undegraded Protein: Postprint

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Abstract

This experiment was conducted to verify the correlation between the content of furosine, an intermediate Maillard reaction product, and the rumen degradation characteristics of heat-processed distillers dried grains with solubles (DDGS) at varying intensities, as well as the intestinal digestibility of rumen undegraded protein, to establish regression equations, and to compare this with acid detergent insoluble protein as a heat-sensitive indicator for feed processing. High-performance liquid chromatography was employed to determine furosine content in differently heat-treated DDGS, the nylon bag method was used to determine rumen degradation characteristics of dry matter and crude protein, and a modified three-step in vitro method was applied to determine intestinal digestibility of rumen undegraded protein, followed by correlation analysis and establishment of regression equations. The results demonstrated: 1) With increasing heating intensity, the contents of furosine and acid detergent insoluble protein in DDGS increased significantly ($P<0.05$), while the rumen effective degradability of dry matter and crude protein and the intestinal digestibility of rumen undegraded protein decreased, with significant differences among treatments ($P<0.05$). 2) Both furosine and acid detergent insoluble protein exhibited significant correlations with the soluble fraction (r=-0.72 vs. -0.60; r=-0.60 vs. -0.51), undegradable fraction (r=0.96 vs. 0.84; r=0.96 vs. 0.85), and effective degradability (r=-0.62 vs. -0.51; r=-0.72 vs. -0.61) of dry matter and crude protein during rumen incubation, as well as with the intestinal digestibility of rumen undegraded protein (r=-0.52 vs. -0.57) ($P<0.05$), enabling the establishment of regression prediction equations. 3) Furosine content demonstrated higher correlation with rumen degradation characteristics compared to acid detergent insoluble protein content; both showed similar correlations with intestinal digestibility of rumen

undegraded protein and total digestible protein content, but furosine content exhibited higher correlation with intestinally digestible protein content (r=0.67). In conclusion, furosine content in differently heat-treated DDGS correlates with rumen degradation characteristics and intestinal digestibility, and regression equations can be established. Furosine content is more suitable than acid detergent insoluble protein content as a key indicator for evaluating the degree of feed heat processing.

Full Text

Correlation between Furosine Content and Ruminal Degradation Characteristics and Intestinal Digestibility of Rumen Undegraded Protein in Distillers Dried Grains with Solubles

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Abstract: This experiment aimed to verify the correlation between furosine content (a Maillard reaction intermediate) and the rumen degradation characteristics and intestinal digestibility of rumen undegraded protein in distillers dried grains with solubles (DDGS) subjected to varying degrees of heat processing, establish regression equations, and compare furosine with acid detergent insoluble protein as a heat treatment sensitivity indicator. Furosine content in differently heat-processed DDGS was determined using high-performance liquid chromatography, rumen degradation characteristics of dry matter and crude protein were measured using the nylon bag method, and intestinal digestibility of rumen undegraded protein was determined using a modified three-step in vitro method. Correlations were analyzed and regression equations were established. The results showed: (1) With increasing heat treatment intensity, furosine and acid detergent insoluble protein contents in DDGS increased significantly ($P < 0.05$), while the effective rumen degradation rate of dry matter and crude protein and the intestinal digestibility of rumen undegraded protein decreased significantly ($P < 0.05$). (2) Both furosine and acid detergent insoluble protein contents were significantly correlated with the soluble fraction ($r = -0.72$ vs. -0.60; $r = -0.60$ vs. -0.51), undegradable fraction ($r = 0.96$ vs. 0.84; $r = 0.96$ vs. 0.85), effective degradation rate ($r = -0.62$ vs. -0.51; $r = -0.72$ vs. -0.61) of dry matter and crude protein, and intestinal digestibility of rumen undegraded protein ($r = -0.52$ vs. -0.57) ($P < 0.05$), enabling the establishment of predictive regression equations. (3) Furosine content showed higher correlation with rumen degradation characteristics than acid detergent insoluble protein content. Both indicators had similar correlations with intestinal digestibility of rumen undegraded protein and total digestible protein content, but furosine content

demonstrated higher correlation with intestinal digestible protein content (r = 0.67). In conclusion, furosine content in heat-processed DDGS correlates with rumen degradation characteristics and intestinal digestibility, allowing for the establishment of regression equations. Furosine content is more suitable than acid detergent insoluble protein content as a key indicator for evaluating feed heat processing degree.

Keywords: furosine; rumen degradation characteristics; intestinal digestibility; DDGS; heat processing

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Distillers dried grains with solubles (DDGS) is a byproduct of ethanol production from corn and other grains, prepared by mixing and drying the filtered residue and evaporated filtrate after solid-liquid separation. When the residue and filtrate are not dried, the product is called wet distillers grains with solubles (WDGS). DDGS contains high levels of protein and serves as an excellent rumen bypass protein feed. With the development of the ethanol industry, DDGS has been widely applied in animal husbandry, particularly in dairy and beef cattle production. Research indicates that drying temperature and duration during DDGS processing significantly affect its quality. Heating induces Maillard reactions, causing substantial variation in nutritional composition and digestibility, generating indigestible and nutritionally worthless products, while markedly reducing available lysine and sugar content and decreasing digestibility. Currently, DDGS quality is primarily evaluated based on color, crude protein (CP), and acid detergent fiber content. However, direct measurement of nutritional components cannot accurately predict rumen degradation characteristics or intestinal digestion of bypass protein. Therefore, identifying indicators that reflect changes in nutritional composition and degradation/digestion during heat processing is critically important.

Evaluating heat damage in feed is essential, as feeding heat-damaged corn gluten meal and DDGS reduces dietary digestibility and inhibits animal growth. Acid detergent insoluble nitrogen has been reported as an indicator for assessing protein heat damage, but studies show it has numerous limitations and cannot quantitatively represent Maillard reaction products. Furosine content has been used to predict available lysine content in rumen undegraded protein, and research has reported correlations between lysine intestinal digestibility and furosine content in DDGS. However, the correlation between furosine content and rumen degradation characteristics of differently heat-processed DDGS has not been reported. During the early stage of Maillard reactions, reducing sugars react with amino groups to form Schiff bases, which then undergo Amadori rearrangement to form protein-bound Amadori compounds—important precursors in the early Maillard reaction. Furosine is produced through acid hydrolysis of Amadori compounds. Widely used in the dairy, food, and grain processing industries as an indicator of processing conditions and quality, furosine has rarely been applied in animal husbandry and feed processing. This study aimed to investigate the correlation between furosine content generated during DDGS heating and

rumen degradation characteristics and intestinal digestibility, establish predictive equations, and compare furosine with acid detergent insoluble protein to provide a reference for using furosine as a new feed testing indicator and for processing and utilization of wet feed materials.

1.1 Sample Collection and Processing

DDGS samples were collected from a feed processing plant in Shuangcheng District, Harbin. The samples were dried in the laboratory at 110, 120, and 130 °C for 0.5, 1.0, and 1.5 hours, yielding nine different heat processing intensities. Samples were ground through a 1 mm sieve for furosine content determination and nutritional component analysis, and through a 2 mm sieve for rumen degradation experiments.

1.2 Experimental Animals and Diet

Three healthy Holstein dairy cows (approximately 600 kg body weight) fitted with permanent rumen fistulas at the Acheng Experimental Base of Northeast Agricultural University were used for rumen degradation experiments. The cows were fed twice daily (08:00 and 16:00) with free access to water. The experimental diet was formulated according to NRC (2001) requirements, with composition and nutrient levels shown in Table 1 .

Table 1 Composition and Nutrient Levels of the Experimental Diet (Air-Dry Basis)

Item	Content
Ingredients	
Chinese wildrye	
Corn silage	
Corn	
Wheat bran	
Molasses beet	
Soybean meal	
Dried distillers grain	
Cottonseed meal	
Corn gluten feed	
Corn germ meal	
Premix[1]	
Total	
Nutrient Levels[2]	
NEL/(MJ/kg)	
CP	
NDF	
ADF	

[1]Contained per kg of premix: VA 8,000,000 IU, VD 700,000 IU, VE 10,000 IU, Fe 1,600 mg, Cu 1,500 mg, Zn 10,000 mg, Mn 3,500 mg, Se 80 mg, I 120 mg, Co 50 mg.
[2]NEL was a calculated value, while other nutrient levels were measured values.

1.3.1 Furosine Content Determination

Samples were hydrolyzed with hydrochloric acid to determine CP content. The hydrolysate was diluted and analyzed by high-performance liquid chromatography (HPLC) with a UV detector at 280 nm using external standard quantification [furosine standard: N- -(2-furoyl-methyl)-L-lysine]. Sample hydrolysate preparation: 0.2 g sample was placed in a sealed heat-resistant test tube with 8 mL of 10.6 mol/L HCl solution and heated at 110 °C for 23 hours. After heating, the hydrolysate was filtered, and the filtrate was used for determination. Two milliliters of sample hydrolysate was taken to measure crude protein content. One milliliter of sample hydrolysate was mixed with 5 mL of 6 g/L ammonium acetate solution, filtered through a 0.22 m aqueous phase filter membrane, and the filtrate was used for HPLC analysis. Chromatographic conditions: C18 silica column (250 mm × 4.6 mm, 5 m particle size) or equivalent; column temperature 32 °C; mobile phase A: 0.1% trifluoroacetic acid solution, mobile phase B: methanol. The chromatographic system was equilibrated with a mixture of mobile phases A and B (50:50) at 1 mL/min flow rate. After baseline stabilization with initial mobile phase, 10 L of 3 mol/L HCl solution was injected to test solvent purity, followed by 10 L of sample solution to determine furosine content. Furosine content was expressed as mass fraction F in mg/kg CP.

1.3.2 Routine Nutritional Component Determination

Dry matter (DM), CP, ether extract (EE), and ash contents were determined according to AOAC methods. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent insoluble protein (NDICP), and acid detergent insoluble protein (ADICP) contents were determined according to Van Soest et al.

1.3.3 Rumen Nylon Bag Method for Determining Rumen Degradation Characteristics of DM and CP

Following the method of Nuez-Ortín et al., approximately 7 g of ground sample was placed in nylon bags (10 cm × 20 cm, 50 m pore size) of known weight, with three replicates per sample. Following the principle of "sequential insertion and simultaneous removal," bags were placed in the rumen fistula and incubated for 0, 2, 4, 8, 12, 24, 36, and 48 hours. No more than 30 bags were placed in the rumen per cow per time point. After removal (including 0 h), bags were rinsed under running water until clear, then dried at 65 °C for 48 hours to constant weight. The total weight of residue and bag was recorded, and residues were ground through a 1 mm sieve, stored in sealed bags, and analyzed for protein content at each time point.

1.3.4 Modified Three-Step In Vitro Method for Intestinal Digestibility

Following the principle and method of Gargallo et al., 7 g of DDGS was placed in nylon bags and incubated in the rumen for 12 hours (four replicates per DDGS per fistulated cow, three cows total). After removal, bags were washed clean and placed in 0.1% methylcellulose solution, oscillated in a 37 °C water bath for 30 minutes, then removed, washed, and dried at 65 °C for 48 hours to constant weight. The CP content of residues was determined. One gram of residue was placed in nylon bags (5 cm × 10 cm) and loaded into Daisy II incubation bottles containing 2 L of pre-warmed HCl solution (pH = 1.9) with 1 g/L pepsin (P-7000, Sigma). Bottles were incubated in an ANKOM Daisy II in vitro fermentation incubator at 39 °C with oscillation for 1 hour. Bags were then removed, washed, and transferred to 2 L of pre-warmed 0.5 mol/L phosphate buffer containing 3 g/L trypsin (P-7545, Sigma) and 50 g/L thymol, and incubated again at 39 °C for 24 hours. After final washing and drying at 65 °C for 48 hours, residues represented simulated post-small intestine digestion samples for CP content determination.

1.4.1 Calculation of Rumen Degradation Parameters

The residual rate (%) of a component in rumen was calculated as: 100 - [100 × (component mass - component mass in residue) / component mass]. Rumen kinetic parameters were calculated using the exponential model proposed by Nuez-Ortín et al.:

$$R(t) = U + D \times e^{-K_d \times (t - T_0)}$$

where $R(t)$ is the residual rate (%) of nutrients after rumen incubation time t; U is the undegradable fraction (%); D is the degradable fraction (%); K_d is the degradation rate of the degradable fraction (%/h); and t is rumen incubation time (h).

Degradation parameters were obtained using the PROC NLIN module in SAS for iterative least squares regression. Effective degradation rate (ED) was calculated as:

$$ED(\%) = S + \left[\frac{D \times K_d}{K_p + K_d}\right]$$

where S is the soluble fraction (%) in rumen culture, $S = 100 - U - D$, and K_p is the rumen outflow rate (0.06/h).

1.4.2 Intestinal Digestibility of Rumen Undegraded Protein

The intestinal digestibility of rumen undegraded protein (dRUP) was calculated as:

$$dRUP(\%) = 100 \times \left[\frac{CP_{12h} - CP_i}{CP_{12h}}\right]$$

where CP_{12h} is the crude protein content (g/kg) in residue after 12 h rumen fermentation, and CP_i is the crude protein content (g/kg) in residue after simulated small intestine digestion.

Intestinal digestible protein (IDP) content was calculated as:

$$IDP(g/kg) = RUCP_{NRC} \times dRUP$$

where $RUCP_{NRC}$ is rumen undegradable protein calculated according to the NRC (2001) model.

Total digestible protein (TDP) content was calculated as:

$$TDP(g/kg) = IDP + EDCP$$

where $EDCP$ is effectively degraded protein content.

1.4.3 Data Statistics and Analysis

Data were analyzed using the PROC GLM module in SAS 9.4 for furosine content, routine nutritional components, rumen degradation parameters, and intestinal digestibility of rumen undegraded protein, with $P < 0.05$ indicating significant differences. The PROC CORR module was used for correlation analysis ($P < 0.05$ indicating significant correlation). The PROC REG module was used for linear regression analysis between furosine content and rumen degradation parameters and intestinal digestibility.

2.1 Effects of Different Heat Processing Degrees on Furosine Content and Routine Nutritional Components in DDGS

As shown in Table 2 , furosine content increased significantly with heating intensity ($P < 0.05$), ranging from 1.16 to 8.10 g/kg CP. Neutral detergent fiber, acid detergent fiber, and acid detergent lignin contents also increased significantly with heating ($P < 0.05$), ranging from 38.89%-47.22% DM, 10.35%-14.29% DM, and 0.71%-1.63% DM, respectively. Crude protein content showed no significant change ($P > 0.05$), but neutral detergent insoluble protein and acid detergent insoluble protein contents increased gradually and significantly ($P < 0.05$), ranging from 8.09%-16.67% CP and 0.93%-3.67% CP, respectively.

Table 2 Effects of Different Degrees of Heat Processing on Furosine Content and Routine Nutritional Compositions in DDGS

Item	110 °C	120 °C	130 °C	P-value
	0.5 h	1.0 h	1.5 h	0.5 h
Furosine (g/kg CP)	1.16^h	2.40^g	3.44^f	2.42^g
DM (% DM)	86.1^e	91.4^d	92.7^bc	84.0^f
OM (% DM)	78.22^d	83.60^c	84.43^c	76.46^e
Ash (% DM)	7.84^d	7.79^d	8.21^a	7.51^e
EE (% DM)	8.20^ab	8.56^a	8.65^a	8.68^a
CP (% DM)				
NDF (% DM)	39.87^d	40.21^d	42.05^c	38.89^e
ADF (% DM)	11.65^c	10.36^d	11.27^c	10.35^d
ADL (% DM)	0.88^de	1.16^bc	0.86^de	0.71^e
NDICP (% CP)	8.09^g	8.67^f	9.48^e	9.08^ef
ADICP (% CP)	0.93^e	1.36^d	1.44^cd	1.44^cd

Values with different letter superscripts in the same row differ significantly ($P < 0.05$), while those with the same or no superscripts do not differ significantly ($P > 0.05$). The same applies to Tables 3 and 4.

2.2 Effects of Different Heat Processing Degrees on Rumen Degradation Characteristics of DM and CP in DDGS

As shown in Table 3 , the soluble fraction of DM in differently heat-processed DDGS ranged from 5.54% to 16.00%, the potentially degradable but insoluble fraction ranged from 80.52% to 87.95%, the degradation rate of the degradable fraction ranged from 2.6 to 3.8 %/h, the undegradable fraction ranged from 0.84% to 8.50%, and the effective degradation rate of DM ranged from 31.1% to 46.8%. With increasing heat treatment intensity, the effective degradation rate of DM decreased gradually, while the undegradable fraction increased gradually, though the potentially degradable fraction and degradation rate showed no consistent trends.

For CP, the soluble fraction ranged from 12.89% to 28.23%, the potentially degradable but insoluble fraction ranged from 68.04% to 81.21%, the degradation rate ranged from 3.1 to 4.5 %/h, the undegradable fraction ranged from 0.72% to 7.84%, and the effective degradation rate ranged from 40.7% to 57.8%. With increasing heat treatment intensity, the soluble fraction and effective degradation rate of CP decreased gradually, while the undegradable fraction increased significantly ($P < 0.05$). Although the potentially degradable fraction and degradation rate varied significantly ($P < 0.05$), they showed no consistent patterns.

Table 3 Effects of Different Degrees of Heat Processing on Rumen Degradation Characteristics of DM and CP in DDGS

Item	110 °C	120 °C	130 °C	P-value
	0.5 h	1.0 h	1.5 h	0.5 h
Rumen Degradation Parameters of DM				
DMS (%)	15.63^a	14.38^ab	14.25^ab	16.00^a
DMD (%)	83.53^bcd	83.90^bc	83.18^bcde	80.63^de
DMU (%)	0.84^i	1.71^h	2.57^g	3.36^f
DMKd (%/h)	3.1^ab	3.2^ab	3.2^ab	3.5^ab
EDDM (%)	44.1^a	43.6^a	43.3^a	46.8^a
Rumen Degradation Parameters of CP				
CPS (%)	28.16^a	28.23^a	28.21^a	27.45^a
CPD (%)	71.12^b	70.33^b	69.63^b	69.64^b
CPU (%)	0.72^h	1.44^g	2.15^f	2.90^e
CPKd (%/h)				
EDCP (%)	58.0^ab	58.5^a	57.7^ab	57.8^ab

2.3 Effects of Different Heat Processing Degrees on Intestinal Digestibility of Rumen Undegraded Protein in DDGS

As shown in Table 4 , different heat processing degrees significantly affected intestinal digestibility of rumen undegraded protein, intestinal digestible protein, and total digestible protein content ($P < 0.05$). Intestinal digestibility of rumen undegraded protein ranged from 90.05% to 93.83%, intestinal digestible protein ranged from 38.6% to 53.5% CP, and total digestible protein ranged from 94.1% to 97.6% CP. With increasing heat treatment intensity, intestinal digestibility of rumen undegraded protein and total digestible protein content decreased gradually, while intestinal digestible protein content increased significantly ($P < 0.05$).

Table 4 Effects of Different Degrees of Heat Processing on Intestinal Digestibility of Rumen Undegraded Protein in DDGS

Item	110 °C	120 °C	130 °C	P-value
	0.5 h	1.0 h	1.5 h	0.5 h
Rumen Degradation Part				
RUPDVE	128.4^d	129.7^cd	134.5^bcd	126.8^d
RUPNRC	115.7^d	116.8^cd	121.2^cd	114.2^d
EDCP	159.5^ab	164.6^a	165.2^ab	156.3^ab
Small Intestine Degradation Part				
dRUP (%)	93.83^a	93.19^ab	92.69^abc	92.73^abc
IDP (% CP)	39.5^b	38.6^b	39.1^b	39.3^b
IDP (g/kg DM)	108.6^bc	108.9^bc	112.4^bc	106.0^c
TDP (% CP)	97.6^a	96.9^a	96.6^a	97.4^a
TDP (g/kg DM)	268.1^c	273.5^b	277.5^a	262.2^e

RUPNRC: Rumen undegradable protein in the NRC (2001) model; RUPDVE: Rumen undegradable protein based on DVE/OEB system. The same applies to Tables 7 and 8.

2.4 Correlations Between Furosine and ADICP Contents and Rumen Degradation Characteristics of DM and CP

As shown in Table 5 , furosine content was significantly negatively correlated with the soluble fraction of DM (r = -0.72, P < 0.05) and effective degradation rate of DM (r = -0.62, P < 0.05), and significantly positively correlated with the undegradable fraction of DM (r = 0.96, P < 0.05). For CP degradation characteristics, furosine content was significantly negatively correlated with the soluble fraction (r = -0.60, P < 0.05), degradation rate (r = -0.62, P < 0.05), and effective degradation rate (r = -0.72, P < 0.05), and significantly positively correlated with the undegradable fraction (r = 0.96, P < 0.05).

ADICP content was significantly negatively correlated with the soluble fraction of DM (r = -0.60, P < 0.05) and effective degradation rate of DM (r = -0.51, P < 0.05), and significantly positively correlated with the undegradable fraction of DM (r = 0.84, P < 0.05). For CP, ADICP content was significantly negatively correlated with the soluble fraction (r = -0.51, P < 0.05), degradation rate (r = -0.51, P < 0.05), and effective degradation rate (r = -0.61, P < 0.05), and significantly positively correlated with the undegradable fraction (r = 0.85, P < 0.05).

Table 5 Correlation Between Furosine and ADICP Contents and Rumen Degradation Characteristics of DM and CP in DDGS with Different Degrees of Heat Processing

Item	DMS	DMD	DMU	EDDM	CPS	CPD	CPU	EDCP
Furosine	<0.0001							
ADICP	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001			

2.5 Correlations Between Furosine and ADICP Contents and Intestinal Digestibility of Rumen Undegraded Protein

As shown in Table 6 , furosine content was significantly positively correlated with rumen undegraded protein predicted by both DVE and NRC models (r = 0.77, P < 0.05; r = 0.77, P < 0.05), with correlation coefficients higher than those for ADICP (r = 0.68, P < 0.05; r = 0.68, P < 0.05). Intestinal digestibility of rumen undegraded protein and total digestible protein (% CP) were significantly negatively correlated with both furosine and ADICP contents, with slightly lower correlation coefficients for furosine (r = -0.52, P < 0.05; r = -0.60, P < 0.05) than for ADICP (r = -0.57, P < 0.05; r = -0.63, P < 0.05). Intestinal digestible protein (% CP) was significantly correlated with

both indicators, showing higher correlation with furosine (r = 0.72, P < 0.05) than with ADICP (r = 0.61, P < 0.05).

Table 6 Correlation Between Furosine and ADICP Contents and Intestinal Digestibility of Rumen Undegraded Protein in DDGS with Different Degrees of Heat Processing

Item	RUPDVE	RUPNRC	dRUP	IDP (% CP)	IDP (g/kg)	TDP (% CP)	TDP (g/kg)
Furosine	<0.0001	<0.0001					
ADICP	<0.0001	<0.0001	<0.0001				

2.6 Regression Relationships Between Furosine/ADICP Contents and Rumen Degradation Characteristics and Intestinal Digestibility

As shown in Tables 7 and 8, furosine content more accurately predicted the soluble fraction, undegradable fraction, effective degradation rate of DM and CP, and intestinal digestible protein (% CP) content than ADICP content. Both indicators showed similar predictive accuracy for intestinal digestibility of rumen undegraded protein and total digestible protein (% CP). Furosine content effectively predicted the potentially degradable but insoluble fraction of DM and the undegradable fraction of CP ($R^2 = 0.95$ and $R^2 = 0.93$, respectively).

Table 7 Linear Regression Between Furosine Content and Rumen Degradation Characteristics and Intestinal Digestibility of Rumen Undegraded Protein in DDGS with Different Degrees of Heat Processing

Predicted Variables (Y)	Prediction Equations (Y = a + bx)	P-value
Rumen Degradation Characteristic		
DMS	Y = 178.08236 - 11.837 × Furosine	<0.0001
DMD	Y = -2.51572 + 9.969 × Furosine	<0.0001
EDDM	Y = 48.40994 - 1.599 × Furosine	
CPS	Y = 319.55224 - 17.188 × Furosine	
CPU	Y = -3.55104 + 9.030 × Furosine	<0.0001
EDCP	Y = 62.80743 - 2.044 × Furosine	<0.0001
RUPDVE	Y = 113.87614 + 6.697 × Furosine	<0.0001
RUPNRC	Y = 102.59111 + 6.033 × Furosine	<0.0001
EDCP	Y = 173.67919 - 5.278 × Furosine	
Intestinal Digestion Characteristics		
dRUP	Y = 93.884 - 0.326 × Furosine	

Predicted Variables (Y)	Prediction Equations (Y = a + bx)	P-value
IDP (% CP)	Y = 35.10433 + 1.698 × Furosine	<0.0001
IDP (g/kg DM)	Y = 96.77796 + 5.117 × Furosine	<0.0001
TDP (% CP)	Y = 97.90431 - 0.367 × Furosine	

Table 8 Linear Regression Between ADICP Content and Rumen Degradation Characteristics and Intestinal Digestibility of Rumen Undegraded Protein in DDGS with Different Degrees of Heat Processing

Predicted Variables (Y)	Prediction Equations (Y = a + bx)	P-value
Rumen Degradation Characteristic		
DMS	Y = 17.87783 - 3.00060 × ADICP	
DMD	Y = -0.36964 + 0.09969 × ADICP	<0.0001
EDDM	Y = 48.68125 - 4.14670 × ADICP	
CPS	Y = 32.03882 - 4.34797 × ADICP	
CPU	Y = -0.50814 + 2.34201 × ADICP	<0.0001
EDCP	Y = 63.32952 - 5.39405 × ADICP	
RUPDVE	Y = 112.29352 + 17.60714 × ADICP	<0.0001
RUPNRC	Y = 101.16534 + 15.86229 × ADICP	<0.0001
EDCP	Y = 175.12574 - 13.98301 × ADICP	
Intestinal Digestion Characteristics		
dRUP	Y = 94.393 - 1.088 × ADICP	
IDP (% CP)	Y = 34.90151 + 4.35899 × ADICP	
IDP (g/kg DM)	Y = 96.23870 + 13.09702 × ADICP	
TDP (% CP)	Y = 98.23709 - 1.09509 × ADICP	

3.1 Effects of Different Heat Processing Degrees on Furosine Content and Routine Nutritional Components in DDGS

The Maillard reaction occurs extensively during food and feed processing, significantly affecting aroma, taste, and nutritional value. Reports indicate that Amadori compounds, intermediate products of the Maillard reaction in dairy products and DDGS, produce a constant proportion of furosine after acid hydrolysis, allowing furosine content to indirectly measure Maillard reaction extent. Higher temperatures and longer heating times increase furosine content during dairy and food processing, a pattern also observed in this experiment. Heat processing also affects nutritional components, increasing neutral and acid detergent fiber contents. Van Soest reported that heating also increases lignin content in forages, which was confirmed by the significant increase in acid detergent lignin

content observed in this study. According to Mckinnon et al., heating does not affect crude protein content, while Zhang et al. found that different heat treatments minimally affected CP content in DDGS but substantially altered protein secondary structure, thereby influencing rumen degradation and intestinal digestion characteristics. In this experiment, CP content showed minimal variation without clear trends across heating intensities, but heat processing significantly affected NDICP and ADICP contents, which increased with heating degree.

3.2 Effects of Different Heat Processing Degrees on Rumen Degradation Characteristics of DM and CP in DDGS

Previous reports indicate that different heating degrees affect rumen degradation characteristics of DM and CP. Heat treatment alters nutrient composition, thereby changing rumen degradation characteristics and effective degradation rates. Studies show that increasing heat intensity gradually reduces 12- and 24-hour disappearance rates of DM and CP. Research on heat-treated whole cottonseed also demonstrated reduced effective rumen degradation rates of DM and CP, consistent with this experiment' s results. The soluble fraction of DM consists of soluble proteins, carbohydrates, and other nutrients. During heating, Maillard reactions transform some soluble substances into the potentially degradable but insoluble fraction and the undegradable fraction. The undegradable protein fraction relates to heat-damaged protein content; more intense heating causes greater protein damage, resulting in largely indigestible and nutritionally worthless products. Therefore, heat treatment significantly affects feed rumen degradation characteristics, and evaluating heat processing not only reflects processing quality but also indicates the degree of nutrient damage.

3.3 Effects of Different Heat Processing Degrees on Intestinal Digestibility of Rumen Undegraded Protein in DDGS

Both raw material type and processing conditions (heating method and intensity) affect protein intestinal digestibility in DDGS. The intestinal digestibility of rumen undegraded protein in DDGS observed in this study was similar to previous research findings. The results demonstrated that within a certain range, higher heating intensity led to lower intestinal digestibility of rumen undegraded protein, consistent with earlier studies. Within the heating range tested, higher heating intensity gradually increased intestinal digestible protein content while decreasing total digestible protein content. Increased heating intensity reduced protein effective degradation rate, resulting in higher rumen undegraded protein content. However, since the reduction in intestinal digestibility of rumen undegraded protein was minimal, the amount of protein digested in the small intestine increased. Increased intestinal digestible protein content can improve milk yield in lactating cows, possibly related to interactions between protein supply and energy metabolism. Studies have shown that excessive heat processing reduces protein intestinal digestibility and total digestible protein content,

which aligns with this experiment' s results.

3.4 Correlations Between Furosine/ADICP Contents and Rumen Degradation Characteristics of DM and CP in DDGS

Feed proteins and fibers form indigestible proteins through acyl-amino reactions or Maillard reactions, known as acid detergent insoluble nitrogen (protein) and neutral detergent insoluble nitrogen (protein). However, acid detergent insoluble nitrogen has many limitations as an indicator of protein heat damage. Heat processing induces Maillard reactions and nutrient heat damage, leading to reduced effective degradation rates of DM. Numerous studies have demonstrated negative correlations between acid detergent insoluble protein and CP rumen degradation rate in both forages and processing byproducts. This experiment confirmed that ADICP content was negatively correlated with effective degradation rates of DM and CP, while furosine content showed stronger negative correlations. During controlled heating, Maillard reactions reduce the content of true soluble protein and soluble carbohydrates in the rumen soluble fraction while increasing the content of acid detergent insoluble protein and lignin in the rumen undegradable fraction. ADICP content was negatively correlated with the soluble fraction of DM and CP, while furosine content showed stronger correlations. ADICP content was positively correlated with the undegradable fraction of DM and CP, while furosine content showed even higher correlations. These results indicate that furosine content better reflects Maillard reaction extent than ADICP content and could serve as a more stable and accurate method for evaluating the soluble and undegradable fractions and effective degradation rates of DM and CP in differently heat-processed DDGS.

3.5 Correlations Between Furosine/ADICP Contents and Intestinal Digestibility of Rumen Undegraded Protein in DDGS

The effects of heat processing on protein intestinal digestibility can be evaluated using acid detergent insoluble nitrogen content. The mobile nylon bag method can study intestinal digestibility of rumen undegraded protein, though this experiment used a modified three-step in vitro method. Studies have reported negative correlations between protein disappearance in the small intestine and entire digestive tract and acid detergent insoluble nitrogen content. Moshtaghi et al. also reported negative correlations between acid detergent insoluble nitrogen and total digestible protein content (% CP) in the entire digestive tract. This experiment confirmed that ADICP content was negatively correlated with intestinal digestibility of rumen undegraded protein and total digestible protein content (% CP). Boucher et al. found that higher levels of furosine, an early Maillard reaction product, resulted in lower lysine intestinal digestibility. Furosine content was also negatively correlated with intestinal digestibility of

rumen undegraded protein and total digestible protein content (% CP), though the correlations were similar to those for ADICP content. However, furosine content showed higher correlation with intestinal digestible protein content (% CP) than ADICP content, suggesting that furosine content can better predict intestinal digestible protein content (% CP), providing a theoretical basis for establishing regression equations.

3.6 Regression Relationships Between Furosine/ADICP Contents and Rumen Degradation Characteristics and Intestinal Digestibility

No previous studies have investigated correlations between furosine content and rumen degradation characteristics. In this experiment, furosine content showed the best fit with the undegradable fractions of DM and CP ($R^2 = 0.95$ and $R^2 = 0.93$, respectively). Furosine content demonstrated better fit with rumen degradation characteristics than ADICP content. Pahm et al. reported correlations between furosine content in rumen undegraded protein and available lysine content in different DDGS samples. Boucher et al. demonstrated that furosine content could predict lysine intestinal digestibility in rumen undegraded protein of DDGS. Mckinnon et al. used acid detergent insoluble nitrogen to predict protein degradation in the small intestine and entire digestive tract, with R^2 values of 0.78 and 0.82, respectively. In this experiment, furosine and ADICP contents showed similar fit with intestinal digestibility and total digestible protein content (% CP), but furosine content demonstrated better fit with intestinal digestible protein content (% CP) than ADICP content. This study represents an initial investigation into using furosine content to predict rumen degradation and intestinal digestion characteristics. The relatively low fit of other predictive equations may be due to the limited scope and depth of research subjects, requiring more extensive experiments to establish more accurate predictive equations.

4 Conclusion

1. Different heat treatments produced significant differences in furosine content, routine nutritional components, rumen degradation characteristics, and intestinal digestibility in DDGS.

2. Both furosine and ADICP contents correlated with rumen degradation characteristics and intestinal digestibility, enabling the establishment of regression equations to predict these parameters.

3. Furosine content more accurately predicted the soluble fraction, undegradable fraction, effective degradation rate of DM and CP, and intestinal digestible protein content than ADICP content. This study preliminarily validates that furosine content can serve as a novel indicator for evaluating feed heat processing degree and assessing the quality and digestive characteristics of heat-processed wet feed materials.

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