Correlation research between gas production characteristics and CNCPS components for roughages

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Correlation research between gas production characteristics and CNCPS components for roughages

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Abstract. The experiment was to study the correlations between gas production characteristics and Cornell Net Carbohydrate and Protein System (CNCPS) components of seven roughages, which shortened the experimental cycles. The ruminant fluid was collected and cultivated in vitro. The results showed negative correlations between cumulative gas productions at 12, 24, 36, 72 h (P < 0.05), 48 h (P < 0.01) and Crude protein (CP), but positive correlations between gas production at 12, 24, 36, 48, 72 h (P < 0.01) and Neutral Detergent Soluble (NDS)/CP, between gas production at 12 h (P< 0.05), 24, 36, 48, 72 h (P < 0.01) and (NDS-ASH(crude ash))/CP. Significant positive correlations were found between parameters a, b, a+b, c and NDS/CP (P< 0.01), as well as between parameters a, b, a+b (P < 0.01), c (P < 0.05) and (NDS-ASH)/CP. However, significant negative correlation was found between b, a+b, c and CP (P < 0.05). The experimental results inferred a positive correlation between degradation rate of Neutral Detergent Fiber (NDF) (P < 0.01), Acid Detergent Fiber (ADF) (P < 0.05) and CP, but negative correlation between degradation rate of NDF (P < 0.05), ADF (P < 0.01) and NDS/CP, (NDS-ASH)/CP.

1. Introduction

The cardinal appraisal procedure for feed nutritional values includes in vitro, in vivo and in-situ methodologies. In Vivo and in situ methodologies are time-consuming and hard sledding, and apparently limited to experimental time and animal quantities when used to evaluate massive samples. Although the CNCPS, which used to reflect comprehensive feed nutritional value, contains plenty of indices and can, its assessment procedure is complex. In Vitro gas production technique, which was first applied by Menke et al [1] successfully, is simple, economical and rapid, and widely used to evaluate feed nutritional values. Seven roughages, which are commonly used in north-eastern China, combined with modified gas production apparatus of In Vitro fermentation, were selected and used to predict gas product volumes, as well as to verify the correlations between gas production characteristics and CNCPS components.

2. Materials and methods

2.1. Experimental design

Four Cannula sheep were used as the source of rumen fluid, which was cultured In Vitro simulating rumen fermentation. Seven roughages used are corn straw silage, Alfalfa, Corn straw, Bean straw, Chinensis Rice, straw, as well as specific silage corn straw.
All experiments were quadruple replicated. The blank control was set at different times for each roughage.

2.2. Estimation of gas production profiles
The rumen fluid was prepared by following procedures: two hours after morning diet, each rumen content was gathered through fistula, and placed promptly into a vacuum flask filled with carbon dioxide (CO₂), finally taken back to the laboratory.

Each rumen content was filtered by four layers gauze. Before fermentation, Menke culture solution was fully filled with CO₂, which indicated by the vanished pink of resazurin. Four hundred mg substrate and sixty mL mixed media was put into each culture flask respectively, then placed into a shaking bath with constant temperature of 39 degree for further culture. Before fermentation, residual gas in each flask due to thermal expansions was tired out.

2.3. Kinetic of gas production
The gas production values of different roughages at different time were plugged into the following formula of gas production model (qrskov et al.,[2]):

\[ p=a+b(1- e^{-\phi t}) \]

where \( t \) is fermentation time, the duration from the beginning of culture to the recording time of gas production, and fixed to 72h in this research; \( p \) is gas production at time.

2.4. Data processing
The experimental data were processed by Excel. The gas production was fitted to the General Linear Model (software SAS V8.1) and compared by analysis of variance. The parameter estimation was carried out by nonlinear regression (NLIN) process; finally the linear correlation and regression analysis was conducted with CORR and REG procedures.

3. Results analysis

3.1. The component of crude protein and carbohydrates in roughages CNCPS
The analysis of dry material (DM), crude fat (EE), and ash (ASH) were carried out according to the method descried in <Feed analysis and quality testing technology>, neutral detergent fiber (NDF), lignin (ADL), neutral detergent insoluble nitrogen(NDLP) and acid washing insoluble nitrogen (ADIP) was conducted with methods developed by Van Soest et al. [3], while the soluble protein (SP) was analyzed by the procedure developed by Krishnamoorthy [4]; analysis of starch was carried out by AACC(American Association of Cereal Chemists) method. The nutritional ingredients of different roughages were shown in table 1.

| Table 1. Nutritional ingredient of roughages. |
|-----------------------------------------------|
| **Corn Straw Silage** | Alfalfa | Corn Straw | Bean Straw | Chinensis | Rice Straw | Specific Silage |
| DM (g/kg) | 91.99±0.27 | 90.10±0.05 | 90.65±0.03 | 88.53±0.05 | 91.84±0.02 | 92.78±0.05 | 96.46±0.06 |
| ASH (%DM) | 6.71±0.05 | 10.22±0.13 | 8.09±0.09 | 5.32±0.93 | 5.72±0.08 | 14.60±0.08 | 6.12±0.22 |
| CP (%DM) | 10.16±0.12 | 20.49±0.15 | 7.35±0.19 | 7.21±0.04 | 8.18±0.26 | 4.73±0.10 | 4.87±0.07 |
| SP (%DM) | 5.41±0.03 | 9.00±0.31 | 3.14±0.08 | 2.92±0.12 | 2.57±0.07 | 1.64±0.05 | 1.82±0.02 |
| ADL (%DM) | 2.39±0.18 | 1.51±0.38 | 5.13±0.29 | 6.86±0.36 | 4.67±0.29 | 1.20±0.14 | 2.80±0.09 |
The results calculation was conducted according to the method used by Sniffen et al. [5]

\[
\text{PA(\%CP)} = \text{NPN(\%SP)} \times 0.01 \times \text{SP(\%CP)}
\]

(2)

\[
\text{PB1(\%CP)} = \text{SP(\%CP)} - \text{PA(\%CP)}
\]

(3)

\[
\text{PC(\%CP)} = \text{ADIP(\%CP)}
\]

(4)

\[
\text{PB3(\%CP)} = \text{NDIP(\%CP)} - \text{ADIP(\%CP)}
\]

(5)

PB2(\%CP)=100−PA(\%CP)−PB1(\%CP)−PB3(\%CP)−PC(\%CP)  (6)

where PA is non-protein nitrogen, PB1 is rapid degrading protein, PB2 is moderate degrading protein, PB3 is slow degrading protein, PC is conjugated protein, CP is crude protein, NPN is non-protein nitrogen. Therefore

\[
\text{CHO(\%DM)} = 100 - \text{CP(\%DM)} - \text{EE(\%DM)} - \text{ASH(\%DM)}
\]

(7)

\[
\text{CC(\%CHO)} = 100 \times [\text{NDF(\%DM)} \times 0.01 \times \text{ADL(\%NDF)} \times 2.4] / \text{CHO(\%DM)}
\]

(8)

\[
\text{CB2(\%CHO)} = 100 \times \left\{ \text{NDF(\%DM)} - \text{NDIP(\%CP)} \times 0.01 \times \text{CP(\%DM)} - \text{NDF(\%DM)} \times 0.01 \times \text{ADL(\%NDF)} \times 2.4 \right\} / \text{CHO(\%DM)}
\]

(9)

\[
\text{CNSC(\%CHO)} = 100 - \text{CB2(\%CHO)} - \text{CC(\%CHO)}
\]

(10)

\[
\text{CB1(\%CHO)} = \text{Starch(\%NSC)} \times [100 - \text{CB2(\%CHO)} - \text{CC(\%CHO)}] / 100
\]

(11)

\[
\text{CA(\%CHO)} = [100 - \text{Starch(\%NSC)}] \times [100 - \text{CB2(\%CHO)} - \text{CC(\%CHO)}] / 100
\]

(12)

where CHO is carbohydrate, NSC is non-structural carbohydrate, CA is saccharide, CB1 is starch and pectin, CB2 is available fiber, CC is non-available fiber. The amount of non-digestible fiber in different roughages was about 2.4 times than that of lignin. CNCPS components of seven roughages were shown in Table 2.

**Table 2.** CNCPS component of seven roughages.

|                | Corn | Straw | Alfalfa | Corn straw | Bean straw | Chinensis | Rice straw | Specific Silage |
|----------------|------|-------|---------|------------|------------|-----------|------------|-----------------|
|                | Silage |       |         |            |            |           |            |                 |
that rapid degradation of carbohydrate scientifically degrading a, NDF b, PB2 c, PA d, CNCPS e degradation rate at different a+b, b c, a

|       | PA(%CP) | PBI(%CP) | PB2(%CP) | PB3(%CP) | PC(%CP) | CA(%CHO) | CB1(%CHO) | CB2(%CHO) | CC(%CHO)          |
|-------|---------|----------|----------|----------|---------|----------|------------|------------|------------------|
|       | 40.96   | 12.32    | 32.93    | 8.79     | 5.01    | 32.41    | 17.78      | 42.59      | 7.23             |
|       | 22.58   | 21.37    | 36.82    | 4.69     | 14.55   | 39.22    | 37.19      | 17.21      | 5.38             |
|       | 25.24   | 17.53    | 32.90    | 16.41    | 7.92    | 23.38    | 11.52      | 50.50      | 14.61            |
|       | 29.95   | 10.61    | 40.66    | 8.73     | 10.06   | 19.38    | 6.62       | 54.94      | 19.05            |
|       | 19.86   | 11.53    | 50.32    | 8.61     | 9.69    | 28.76    | 11.45      | 46.35      | 13.43            |
|       | 21.70   | 13.00    | 36.29    | 17.74    | 11.28   | 8.93     | 5.58       | 82.88      | 3.62             |
|       | 29.26   | 8.18     | 36.16    | 14.29    | 12.10   | 15.97    | 10.38      | 66.05      | 7.61             |

CNCPS divides feed carbohydrate into CA (rapid degrading component), CB2+ CB1 (moderate degrading component) and CC (non-degrading component). The CA value from alfalfa was scientifically higher than the other groups, which demonstrated a higher CA content of available carbohydrate in alfalfa and a better nutritional quality. The CA value of corn straw silage was slightly lower than that of alfalfa, but CB2+CB1 value was high, which also indicated a good nutritional quality.

3.2. Gas production profiles with in Vitro formation and NDF, ADF degradation rate

The gas production characteristics of 7 roughages after 72h in Vitro fermentation and NDF, ADF degradation rate at 48h were shown in table 3.

**Table 3.** Gas production characteristics and NDF, ADF degradation rate at 48h of seven roughages.

|       | Corn Straw Silage | Alfalfa | Corn Straw | Bean Straw | Chinensis | Rice Straw | Specific Silage | Cor Straw |
|-------|-------------------|---------|------------|------------|-----------|------------|----------------|-----------|
| 12h(ml) | 17.98±1.02| 12.03±1.2 | 23.28±0.5 | 27.47±3.8 | 21.19±0.9 | 38.17±0.3 | 31.81±0.9 |
| 24h(ml) | 22.81±0.47| 21.63±1.5 | 35.27±1.3 | 36.27±4.5 | 33.14±1.0 | 50.51±1.5 | 49.79±1.2 |
| 36h(ml) | 36.63±0.6 | 36.25±0.6 | 45.24±0.7 | 47.22±4.1 | 45.18±2.8 | 55.64±0.4 | 52.66±2.0 |
| 48h(ml) | 36.63±0.6 | 37.27±1.3 | 48.32±0.9 | 50.83±5.2 | 45.29±2.6 | 55.89±0.6 | 53.97±3.0 |
| 72h(ml) | 43.73±1.92| 40.95±2.1 | 49.98±1.0 | 51.70±5.8 | 48.51±3.0 | 56.07±1.2 | 55.52±3.5 |
| a       | 1.96±0.15| 1.51±0.29 | 2.35±0.04 | 4.18±0.27 | 2.33±0.36 | 6.56±0.13 | 5.12±0.28 |
| b       | 53.13±0.55| 50.62±0.2 | 58.72±0.9 | 62.50±0.2 | 55.18±0.3 | 66.40±0.3 | 63.39±0.4 |
| c       | 55.46±0.15| 52.13±0.3 | 60.42±0.5 | 66.68±0.2 | 57.04±0.6 | 72.96±0.3 | 68.51±0.2 |
| NDF degradation rate (%) | 39.75±1.23| 44.64±1.7 | 37.77±0.8 | 36.82±0.5 | 38.07±5.3 | 28.64±0.4 | 35.34±0.4 |
| ADF Degradation rate (%) | 20.69±0.92| 22.55±0.9 | 19.86±0.4 | 19.29±0.7 | 20.38±1.9 | 18.29±0.0 | 18.94±0.0 |

a, b, c, d, e; different letters of data superscripts in same row mean different significantly (P <0.05)

As shown in Table 3, rice straw had a highest a (gas production parameter) value, which mean the rapid degradation component in rice straw could generate most gas production quantity, and more than that of the other roughages (P< 0.05). Gas production of slow degradation component from rice straw
was significantly higher than that of bean straw and specific silage corn straw, with lowest value from alfalfa. Meanwhile, rice straw had the highest potential gas production (a+b) and gas production constant (c) value, which were significantly higher than the other 6 roughages (P<0.05).

3.3. Correlations between different nutritional ingredients and gas productions with time and 48h NDF, ADF degradation rate

The correlations between different nutritional ingredients and gas productions with time and 48h NDF, ADF degradation rate were shown in Table 4.

Table 4. Correlations between nutrition, gas productions with time and NDF, ADF degradation rate at 48h.

|         | 12h  | 24h  | 36h  | 48h  | 72h  | NDF Degradation Rate | ADF Degradation Rate | a   | b   | a+b  | c  |
|---------|------|------|------|------|------|-----------------------|-----------------------|-----|-----|------|----|
| CP      | -    | 0.87*| -    | 0.89*| -    | 0.93**               | 0.96**               | -   | 0.86*| -    | -  |
| ADF     | 0.81 | 0.85*| 0.90*| 0.88*| 0.85*| -0.89*               | -0.89*               | 0.73| 0.78 | 0.76 | 0.90*|
| NDF     | 0.67 | 0.71 | 0.70 | 0.75 | 0.66 | -0.79                | -0.66                | 0.52| 0.59 | 0.58 | 0.59|
| NDS/C   | 0.95*| 0.94*| 0.96*| 0.93**| 0.94*| -0.91*               | -0.95**              | 0.97*| 0.97*| 0.97*| 0.96*|
| CP      | -    | -    | -    | -    | -    | 0.98*                | -                     | 0.97*| 0.97*| 0.97*| 0.96*|
| NDS     | -0.67| -0.71| -0.70| -0.75| -0.66| 0.79                 | 0.66                 | -0.52| -0.59| -0.58| -0.59|
| HC      | 0.52 | 0.54 | 0.51 | 0.58 | 0.47 | -0.63               | -0.46                | 0.35| 0.42 | 0.41 | 0.36|
| (NDS/ASH)/CP | 0.92*| 0.88*| 0.92*| 0.95**| 0.96*| -0.93**              | -0.87*               | 0.92*| 0.98*| 0.96*| 0.91*|

*: Correlation, **: Significant correlation.

3.4. Regression model between gas production at 12, 24, 36, 48, 72h of different roughages and components of 400 mg substrate

Absolute amounts of nutrition in carbohydrate and protein according to CNCPS (400 mg) were shown in table 5.

Table 5. Absolute amounts of nutrition in carbohydrate and protein according to CNCPS (400 mg)

|         | Corn Straw Silage | Alfalfa Corn Straw | Bean Straw | Chinensis Corn Straw | Rice Straw | Specific Silage Corn Straw |
|---------|-------------------|-------------------|------------|----------------------|------------|---------------------------|
| PA(mg)  | 1.56              | 1.67              | 0.98       | 0.79                 | 1.06       | 0.38                      | 0.53 |
| PB1(mg) | 0.81              | 1.58              | 0.52       | 0.47                 | 0.55       | 0.23                      | 0.24 |
| PB2(mg) | 2.12              | 2.72              | 1.39       | 1.22                 | 1.51       | 0.64                      | 0.68 |
| CA(mg)  | 10.41             | 11.58             | 6.81       | 5.77                 | 7.06       | 2.64                      | 3.36 |
| CB1(mg) | 5.20              | 11.68             | 3.27       | 2.97                 | 3.49       | 1.42                      | 1.50 |
| CB2(mg) | 12.52             | 6.50              | 15.06      | 16.34                | 14.33      | 24.29                     | 22.47|

Multiple regression analysis were conducted between the gas producing ingredients after fermentation, PA, PB1, PB2, CA, CB1, CB2, and gas production after different in Vitro culture
durations, and we could inferred the regression model between gas production (Yt, mL) under time t and nutritional ingredients of different roughages, as shown below:

\[ Y_{t2} = 144.44\, PA+1844.76PB1-114.15\, PB2-41.58CA-204.07CB1+31.6 \quad (R^2 = 0.973 , \, n = 5) (13) \]
\[ Y_{t3} = 165.35PA +1211.32PB1-127.76PB2-31CA-130.08CB1+57.39 \quad (R^2 = 0.974 , \, n = 5) (14) \]
\[ Y_{t6} = 79.62PA+753.99PB1-44.58PB2-21.33CA-83.49CB1+55.36 \quad (R^2 = 0.988 , \, n = 5) (15) \]
\[ Y_{t8} = 40.77PA+726.42PB1-64.63PB2-11.26CA-79.61CB1+57.45 \quad (R^2 = 0.989 , \, n = 5) (16) \]

\[ Y_{t2} = -15.68 \, PA-77.75 \, PB1 - 4.01 \, PB2 + 2.89 \, CA + 9.02 \, CB1+62.02 \quad (R^2 = 0.991 , \, n = 5) (17) \]

The feeds selected for modeling were alfalfa, corn straw, bean straw, chinensis and rice straw. The remained feeds (corn straw silage and specific silage corn straw) were used to validate the model. The results were shown in Table 6.

| Table 6. Validation of regression model. |
|-----------------------------------------|
| **Corn Straw Silage** | **Specific Silage Corn Straw** |
| Measured Data | Predicted Data | Deviation(%) | Measured data | Predicted Data | Deviation(%) |
| PA | 1.56 | 1.48 | 5.13 | 0.53 | 0.50 | 5.66 |
| PB1 | 0.81 | 0.82 | 1.23 | 0.24 | 0.24 | 0.00 |
| PB2 | 2.12 | 2.12 | 0.00 | 0.68 | 0.69 | 1.47 |
| CA | 10.41 | 10.41 | 0.00 | 3.36 | 3.32 | 1.19 |
| CB1 | 5.20 | 5.22 | 0.39 | 1.50 | 1.46 | 2.67 |

Deviation = |(data of prediction-data of measure)/data of measuring|×100%

4. Discussions

4.1. Analysis of parameter patterns
In this research, all the parameters, a, b, a+b and c in the model p=a+b (1-e^-t) had real biological implication and showed certain regularity. Parameter a was the intercept on axis Y of the cumulative gas production curve when t= 0, which also represented the instant gas production of rapid degradation components (the soluble component) of feeds; parameter b represented gas production of insoluble component but fermentable components (slow degradation component) in feed; parameter c was the gas production constant during whole fermentation, which also represented the consumption rates of potential digestive components per hour; parameter a+b was the potential gas production volume. The rice straw in table 2 had the highest a+b and c values among seven roughages.

4.2. Correlation between gas production characteristic and ingredients
It was reported the gas production of roughages originated from carbohydrate fermentation [6]. The accumulative gas production of roughages in Vitro fermentation was dominated by the ratio between soluble non-structural carbohydrate and crude proteins [7-8]. The higher ratio infers a higher accumulative gas production and increasing in Vitro fermentation. Tan et al [9] inferred an appropriate ratio between non-structural carbohydrate and structural carbohydrate in sheep diet. If so, the ratio could benefit degradation and digestion of fibers in gastrointestinal tract. These results also validated our research.

4.3. Verification of regression model by prediction results between gas production and roughages nutrition
It could be speculated from the regression model that, gas production at different times were mainly depending on contents of PA, PB1, PB2, CA and CB1, less impacted by CB2 in roughages.

The deviation of predicted results from the regression model between gas production and roughages nutrition in table 5 were less than 1.23%, 1.47%, 1.19%, 2.67% for PB1, PB2, CA, CB1, respectively; however, the deviation for PA was 5.66%. The determination of PA was the difference between crude and true protein. The latter was greatly impacted by the poor repetitiveness of quantitative filter paper used.

The CNCPS methodology is manpower and resource consuming [10-11], however the determination time is long. Using in Vitro batch fermentation for modelling rumen to predict gas production for each roughage is simple and practical, and has significant positive correlations with CNCPS methodology for prediction of diet nutrition [12]. Therefore, this research was used in Vitro batch fermentation methodology to determine the gas production of seven roughages at different times. The values from five roughages were adopted to establish the regression model between gas production and diet nutrition, the remained two roughages values were used to validate and calibrate the model, which was beneficial for practical operation.

Whether the regression model established in this research could be used to predict rumen gas production for single or mixed diet still needs further verification by in Vivo results. However, the regression model for sorting nutrition content is practical.

5. Conclusions
This research verified significant correlation between gas production characteristics, NDF, ADF degradation rate for each roughage and CP, NDS/CP, (NDS-Ash)/CP. This verified in Vitro gas production methodology could be used to evaluate the digestibility of nutrient. Combined with fermentation parameters of artificial rumen, this methodology could be used to evaluate diet digestibly accurately, even guide the use of these roughages scientifically.

The regression model developed in this research was time-saving and could be used to predict CNCPS nutrient of each roughage. However, the roughages were often fed in combination with no less than two different roughages, therefore further verification for mixed roughages should be conducted.

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