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## ***In vitro* effect of non-fiber carbohydrates on gas production parameters of various fibrous feeds**

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### **Abstract**

The aim of the present study was to evaluate the effect of various non-fiber carbohydrates on gas production parameters of fibrous feeds [alfalfa hay (AH), wheat straw (WS) and cottonseed hulls (CH)]. Treatments were AH, WS and CH as non-supplemented or supplemented with glucose (GLU), fructose (FRU), sucrose (SUC) and starch (STA) used as 0.0, 30, 60 and 90 mg/g DM in a completely randomized design with a 3x4x4 factorial arrangement. *In vitro* gas production parameters of the treatments were determined (4 runs). Approximately, 0.3 g of each sample was placed in a 100 ml glass syringe containing 40 ml of buffered rumen fluid (ratio of buffer to rumen fluid was 2: 1). Rumen fluid was obtained from two rumen cannulated sheep (45.5± 2 kg, body weight) before the morning feeding and immediately strained through four layers of cheesecloth. Animals were fed with 1.5 kg DM alfalfa hay and 0.4 kg DM concentrates (165 g CP/ kg DM) per head per day. Syringes were incubated at 39 °C and the volume of gas produced was recorded at 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. The gas production data were fitted to an exponential equation of  $P = b (1 - e^{-ct})$ , where  $b$  is the volume of gas produced,  $c$  is the fractional rate constant of gas production (/h),  $t$  is the incubation time (h) and  $P$  is the volume of gas produced at time  $t$ . Statistical analysis was conducted using SAS procedure. Supplementation of AH, CH and WS with each source of NFC, particularly as 60 and 90 mg/g DM, caused to increase the volume of gas produced ( $b$ ) significantly ( $P < 0.05$ ). Supplementation of AH with the source of NFC used in this study as 60 and 90 mg/g DM increased significantly ( $P < 0.05$ ) the fractional rate constant of gas production ranged from 11 to 33%. When wheat straw was supplemented with FRU as 60 and 90 mg/g DM and SUC as 60 mg/g DM the parameter of ( $c$ ) was significantly ( $P < 0.05$ ) increased from 0.024 to 0.030, 0.031 and 0.029, respectively. Supplementation of CH with FRU and SUC as 30 mg/g DM caused to decrease significantly ( $P < 0.05$ ) the  $c$  parameter from 0.017 to 0.010 and 0.011, respectively. It might be concluded that each source of NFC evaluated in the present study caused unique pattern of fermentation when added to a fibrous material.

**Keywords:** non fiber carbohydrates, gas production, fibrous feeds

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## Summary

The aim of the present study was to evaluate the effect of various non-fiber carbohydrates on gas production parameters of fibrous feeds [alfalfa hay (AH), wheat straw (WS) and cottonseed hulls (CH)]. Treatments were AH, WS and CH as non-supplemented or supplemented with glucose (GLU), fructose (FRU), sucrose (SUC) and starch (STA) used as 0.0, 30, 60 and 90 mg/g DM in a completely randomized design with a 3x4x4 factorial arrangement. In vitro gas production parameters of the treatments were determined (4 runs). Approximately, 0.3 g of each sample was placed in a 100 ml glass syringe containing 40 ml of buffered rumen fluid (ratio of buffer to rumen fluid was 2: 1). Rumen fluid was obtained from two rumen cannulated sheep (45.5± 2 kg, body weight) before the morning feeding and immediately strained through four layers of cheesecloth. Animals were fed with 1.5 kg DM alfalfa hay and 0.4 kg DM concentrates (165 g CP/ kg DM) per head per day. Syringes were incubated at 39 °C and the volume of gas produced was recorded at 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. The gas production data were fitted to an exponential equation of  $P = b(1 - e^{-ct})$ , where  $b$  is the volume of gas produced,  $c$  is the fractional rate constant of gas production (/h),  $t$  is the incubation time (h) and  $P$  is the volume of gas produced at time  $t$ . Statistical analysis was conducted using SAS procedure. Supplementation of AH, CH and WS with each source of NFC, particularly as 60 and 90 mg/g DM, caused to increase the volume of gas produced ( $b$ ) significantly ( $P < 0.05$ ). Supplementation of AH with the source of NFC used in this study as 60 and 90 mg/g DM increased significantly ( $P < 0.05$ ) the fractional rate constant of gas production ranged from 11 to 33%. When wheat straw was supplemented with FRU as 60 and 90 mg/g DM and SUC as 60 mg/g DM the parameter of ( $c$ ) was significantly ( $P < 0.05$ ) increased from 0.024 to 0.030, 0.031 and 0.029, respectively. Supplementation of CH with FRU and SUC as 30 mg/g DM caused to decrease significantly ( $P < 0.05$ ) the  $c$  parameter from 0.017 to 0.010 and 0.011, respectively. It might be concluded that each source of NFC evaluated in the present study caused unique pattern of fermentation when added to a fibrous material.

*Keywords: non fiber carbohydrates, gas production, fibrous feeds*

## Introduction

Non-fiber carbohydrates (NFC) are an important source of energy in the rations of high producing ruminants, however, they vary in their effects on the animal performance (Ariza et al., 2001). Understanding how the array of NFC fit within the total ration picture, and how they differ in the nutrients they supply to the animal, will give a better sense of how we should use them in ration formulation. Differences among NFC components regarding microbial fermentation may also imply that the complement of NFCs in a particular feedstuff

is important when predicting animal response.

Several methods such as *in vivo*, *in situ* and *in vitro* techniques have been used in order to evaluate the nutritive value of feedstuffs. The *in vitro* gas production technique has proved to be a potentially useful technique for feed evaluation (Getachew et al., 2004) as it is capable of measuring rate and extent of nutrient degradation (Cone et al., 2002). In addition, *in vitro* gas production technique provide less expensive, easily to determine (Getachew et al., 2004) and suitable for use in developing countries (Chumpawadee et al., 2005).

The objective of this study was to evaluate the effect of various non-fiber carbohydrates on gas production parameters of fibrous feeds [alfalfa hay (AH), wheat straw (WS) and cottonseed hulls (CH)].

## Materials and Methods

Samples of AH and CH were ground through a 2 mm screen and WS through a 1 mm screen, then they were dried in an oven at 66 °C for 48 h. Dry Matter (DM) was determined by drying the samples in a forced air oven at 66°C for 48h and ashing the samples in a muffle furnace at 550°C for 3 h (AOAC, 2002). Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein was calculated as  $N \times 6.25$ . Ether extract (EE) was determined by the method of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the methods of Van Soest et al (1991). Treatments were AH, WS and CH as non-supplemented or supplemented with glucose (GLU), fructose (FRU), sucrose (SUC) and starch (STA) used as 0.0, 30, 60 and 90 mg/g DM in a completely randomized design with a 3x4x4 factorial arrangement. *In vitro* gas production parameters of the treatments were determined using the Menke and Steingass (1988) procedure. Rumen fluid was obtained from two rumen cannulated sheep (45.5± 2 kg, body weight) before the morning feed and immediately strained through four layers of cheesecloth. Animals were fed once daily at the maintenance level with 1.5 kg DM alfalfa hay and 0.4 kg DM concentrates (165 g CP/ kg DM) per head per day. Approximately 0.3 g of sample (4 runs) was placed in a 100 ml glass syringe. The syringes were prewarmed at 39 °C before the injection of 40 ml of buffered rumen fluid (ratio of buffer to rumen fluid was 2: 1) into each syringe followed by incubation in a water bath at 39 °C. The volume of gas produced was recorded at 2, 4, 8, 12, 24, 36, 48, 72 and 96 h after incubation. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to an exponential equation of  $P = b(1 - e^{-ct})$ , where  $b$  is the volume of gas produced,  $c$  is the fractional rate constant of gas production (/h),  $t$  is the incubation time (h) and  $P$  is the volume of gas produced at time  $t$ . All of the data were analyzed by using software of SAS (2003) and the gas production parameters of the supplemented samples were compared with experimental fibrous feeds as control using dunnet's test at  $p < 0.05$ .

## Results and Discussion

Chemical composition of experimental fibrous feeds are shown in Table1.

The volume of gas produced from WS was significantly higher than that of AH and CH ( $P < 0.05$ ). Also, WS had greater fractional rate constant of gas production compared with CH but less than AH ( $P < 0.05$ ). Getachew et al. (2004) reported that strong negative correlations occurred between CP content of feeds and potential gas production ( $b$ ). However, neither CP nor NDF level was correlated with rate of gas production, which was in agreement with present findings.

**Table 1. Chemical composition of experimental fibrous feeds**

Item	Alfalfa hay	Wheat straw	Cottonseed hulls
DM (%)	90	87.8	89.9
CP (%)	17.7	3.1	5
NDF (%)	46.3	83.8	86.5
ADF (%)	37.3	57.7	67.8
EE (%)	2.3	0.6	1.8
ASH (%)	8.1	3.6	7.8

DM=dry matter; CP=crude protein; NDF=neutral detergent fiber; ADF=acid detergent fiber; EE=ether extract

**Table 2. Gas production parameters of alfalfa hay (AH), wheat straw (WS) and cottonseed hulls (CH) as non supplemented or supplemented with non-fiber carbohydrates (NFC)**

Treatments	Level of NFC (mg/g DM)	Gas Production Parameters					
		b			c		
		AH	WS	CH	AH	WS	CH
Without NFC(control)	0	72.14	86.74	72.62	0.070	0.024	0.017
GLU	30	91.89 *	90.86	80.37	0.075	0.02	0.014
	60	89.13 *	92.97*	84.5 *	0.078 *	0.023	0.018
	90	96.77 *	91.19	85.11 *	0.082 *	0.025	0.019
FRU	30	95.75 *	97.41 *	81.94	0.076	0.028	0.010 *
	60	101.98 *	99.65 *	89.72 *	0.089 *	0.030 *	0.015
	90	87.63 *	106.59 *	77.83	0.084 *	0.031 *	0.017
STA	30	99.89 *	86.49	91.26 *	0.075	0.021	0.018
	60	103.2 *	94.35 *	90.65 *	0.081 *	0.021	0.017
	90	108.28 *	100.82*	89.26 *	0.082 *	0.023	0.017
SUC	30	93.08 *	89.62	81.53	0.075	0.027	0.011 *
	60	113.27 *	107.3*	72.78	0.091 *	0.029 *	0.015
	90	111.77 *	102.81 *	82.84 *	0.097 *	0.029	0.016
s.e.m		2.33	3.29	5.06	0.006	0.002	0.002
P-value		< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

\* means with\* in each column have significantly difference with control (P< 0.05).

b= volume of gas produced; c= fractional rate constant of gas production

The effect of NFC on in vitro gas production parameters (b and c) of fibrous feeds evaluated in the present study are shown in Table 2. The volume (b) and the rate constant of gas produced (c) from AH was significantly (P< 0.05) increased by the adding of NFC sources, particularly as 60 and 90 mg/g DM. Supplementation of WS with FRU, GLU (60 mg/g DM), STA and SUC (60 and 90 mg/g DM) caused to a significant increase in the volume of gas produced. The fractional rate constant of gas production was significantly (P< 0.05) increased when FRU and SUC was added to WS. In CH as a fibrous feed, STA at the third applied levels, GLU (60 and 90 mg/g DM), FRU (60 mg/g DM) and SUC (90 mg/g DM) significantly caused to increase b, but FRU and SUC as 30 mg/g DM caused to reduce c (P< 0.05).

## Conclusions

These data indicate that the fermentability of WS was altered by FRU and SUC

supplementation. In addition, the results indicate that NFC sources as 60 and 90 mg/g DM had potential to increase the fermentability of AH, but none of the NFC sources altered the fermentability of CH. It might be concluded that each source of NFC evaluated in the present study caused unique pattern of fermentation when added to a fibrous material. In addition, different effects of a specific source of NFC on different fibrous feeds might demonstrate interaction effects between NFC and feedstuffs type. Therefore, it can be concluded that the soluble fraction of fibrous feeds greatly contributes to gas production.

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