Effect of soybean molasses-adsorbents on in vitro ruminal fermentation characteristics, milk production performance in lactating dairy cows

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Running title: Soybean molasses-adsorbents

Abstract: This study aimed to evaluate the *in vitro* fermentation characteristics of corncob powder (CRP), wheat bran (WB)**,** rice husk (RH)**,** defatted bran (DB) and soybean hulls (SH) when mixed with soybean molasses at a ratio of 30:100 (dry matter basis), using a batch culture incubation. During *in*

vitro study, SH showed better fermentation characteristics (including greater maximum gas production, shorter time to achieve half of *Vf*, greater concentrations of acetate, propionate and total VFA, and less initial fractional rate of degradation) than other four substrates, while WB had the greatest values of DM and NDF disappearance, NH3-N and butyrate concentrations among substrates. A randomized complete block designed *in vivo* experiment was conducted with 24 Holstein cows (534 ± 58 kg BW, 2.8 ± 0.7 parity, 129 ± 23 d in milk) randomly assigned to three experimental diets: Control, WB (WB adsorbed to soybean molasses replaced 150 g of corn meal per 1000 g of diet dry matter [DM]) or SH (SH adsorbed to soybean molasses replaced 100 g of wheat bran and 50 g corn meal per 1000 g of diet DM). The results indicated that cows received WB diet had greater (P<0.01) milk fat and total milk solid content than cows fed control and SH diets, and cows received WB and SH diets tended to have greater (P<0.01) milk protein content and blood glutamic-pyruvic transaminase concentration than cows fed control diet. Furtherly, cows received WB diet had greater (P<0.01) blood amylase and lactate dehydrogenase concentration than that of cows fed control diet during middle lactation.

In conclusion, dietary supplementation of molasses adsorbed by-products like WB and SH have positive effect on promoting rumen fermentation, milk quality and blood metabolism in early- and middle-lactating dairy cows. The results offered a new products and feeding way in dairy farming

Keywords: blood metabolites; dairy cow; milk production; molasses; ruminal fermentation

Introduction

Soybean molasses is a by-product of soybean meal concentrate. The molasses byproduct results from the separation of solids and the evaporation of ethanol from the liquid fraction during the ethanol extraction processes of concentrated soybean meal. It is rich in oligosaccharides, saponins, isoflavones and other phytochemicals (Shi et al. 2013). Most of the carbohydrates can be fermented rapidly in the rumen by the microbes as energy sources, leading to efficient utilization of the rapidly degradable nitrogen fraction and greater microbial protein synthesis. The net result can be increased milk protein production.

Previous studies mainly focused on molasses extracted from sugarcane and beet, which have been widely used in the animal feed industry. Molasses is a sugar-containing liquid feed that can increase the ruminal fermentability of the diet, while stimulating dry matter intake (DMI) (Firkins et al., 2008). It also serves as a fat carrier in a diet and is used to enhance mixing of ingredients to prevent sorting (Murphy et al., 1997). Feeding a sugar-based product can change the ruminal fermentation pattern, decrease ruminal ammonia (NH_3) concentration in dairy cows (Broderick and Radloff, 2004; Broderick et al., 2008), and increase ruminal butyrate concentration (Hristov and Ropp, 2003; DeFrain et al., 2006). It is well-known that sugars can be rapidly fermented in the rumen, theoretically leading to lactic acid production and a decline in ruminal pH, which potentially depresses fiber digestibility (Oelker et al., 2009). Martel et al. (2011) reported that dietary supplementation with cane molasses affected volatile fatty acid (VFA) concentration, milk production, and milk fat and protein yields. Furthermore, supplementation of blended molasses (50% beet sugar molasses and 50% yeast molasses) can alleviate the decrease of feed intake, and increase milk production and milk protein content in dairy cows during heat stress (Zhang, et al., 2013). Broderick and Radloff (2004) reported that replacing

high-moisture corn with molasses improved fiber digestibility, likely reflecting a stimulatory effect of molasses on fiber-digesting ruminal bacteria.

Our first hypothesis for this study using a batch culture *in vitro* fermentation technique was that WB and SH had better *in vitro* fermentation characteristics among five different feeds adsorbed to soybean molasses. and our second hypothesis for this study using lactating dairy as the experimental animals was that WB and SH have positive effect on improving milk quality and promoting blood metabolism in lactating dairy cows.

Material & Methods

The experiments were conducted according to the animal care guidelines of the Animal Care Committee, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha City, Hunan Province, China (No. KYNEAAM-2006-0015).

1.1 *In vitro experiment*

1.1.1 Fermentation substrates

Corncob powder (CRP), wheat bran (WB), rice husk (RH), defatted bran (DB) and soybean hulls (SH) were mixed with soybean molasses at a ratio of 100:30 (DM basis), dried at 65ºC for 24 h, ground through a 1-mm sieve and stored in an airtight bag until further assays(Offered by Fengyi (Shanghai) biotechnology research and development center co. LTD. Shanghai, 200137, China). The chemical compositions of the five soybean molasses-adsorbents are listed in Table 1.

1.1.2 In vitro gas production and sampling

The *in vitro* study was designed as a single factor randomized block design to evaluate the effects of five molasses-adsorbents. *In vitro* batch culture solutions were prepared using macroelement solution, buffer and reducing agent. The buffer was prepared as described by Tang et al. (2006) and it was kept

anaerobic by continuously pumping carbon dioxide for 2 h. Rumen fluid was obtained from three rumen-cannulated Holstein dairy cows fed *ad libitum* a mixed diet of rice straw and concentrate (60:40, wt/wt). The diets were offered twice daily at 0500 and 1600 h. Rumen contents of each dairy cow were obtained from various locations within the rumen immediately before the morning feeding, mixed and strained through four layers of cheesecloth under a continuous CO_2 stream. The obtained rumen fluid was then anaerobically combined with *In vitro* batch culture solutions in the proportion of 1 to 9 at 39ºC.

A 1000 ± 3 mg sample of each substrate was accurately weighed into a 100 mL fermentation bottle (Wanhong Glass Instrument Factory, China) pre-warmed at 39ºC, then 50 ml of the mixed fluids (rumen fluids:artificial saliva = 1:9, V/V) were dispensed into each bottle. Each sample was replicated three times at each incubation time point. Bottles containing only mixed fluids were incubated as blanks together with the bottles containing different molasses-adsorbents. All fermentation bottles were connected with pressure sensors (CYG130-12, SQ sensor, China) and incubated at 39ºC. The pressure in all the bottles was recorded at 0, 1, 2, 4, 6, 12, 24, and 48 h during the process of *in vitro* fermentation. Three bottles for each treatment were removed from the incubator to stop the incubation and the pH of the fluid in each bottle was determined immediately. The undegraded residues were filtered through 2 layers of nylon cloth (40-µm pore size). The incubation fluid was sampled at 12, 24 and 48 h for determination of NH_3-N and VFA concentrations.

1.2 In vivo experiment

1.2.1 Experimental diets and design

A randomized complete block design was with 24 multiparous Holstein cows (534 ± 58 kg BW, 2.8 ± 0.7 parity) blocked into 8 blocks to ensure equal numbers of early-lactation (0-100 d) and mid-lactation

106 (100-200 d) cows for each treatment. One cow per group was randomly assigned to one of three
107 treatments：Control (basal diet); WB (WB adsorbed to soybean molasses replaced 150 g of corn meal
108 per 1000 g of diet dry matter [DM]) or SH (SH adsorbed to soybean molasses replaced 100 g of wheat
109 bran and 50 g corn meal per 1000 g of diet DM). The three experimental diets were formulated to meet
110 the nutrient requirements of lactating cows according to NRC (2001), and the treatmetns were chosen
111 based on the *in vitro* fermentation results (Table 2).
112 The experiment lasted 5 weeks. Throughout the trial, cows were housed in a tie-stall facility. Diets
113 were offered adlibitum twice daily at 0500 and 1600 h, and had free access to clean water. Before
114 starting the experiment, all cows were fed the same diets for 2-wk.
115 1.3 Sample collection and handling
116 The experimental diets were offered twice daily, the orts were collected and recorded once daily.
117 Weekly composites of the concentrates, rice straw, orts, **DGS** (distillers grains with solubles) and beet
118 pulp were obtained from daily samples of about 0.5 kg and stored at -20ºC until analysis. Cows were
119 milked twice daily, and individual milk yield was recorded at each milking during
120 5-weeks-expreriment. Milk samples were collected at 2 consecutive (p.m. and a.m.) milkings midway
121 through wk 5 of the experimental phase for conventional analysis. Concentrations and yields of fat,
122 protein, lactose, total solids (**TS**) and solids-not-fat (**SNF**) were computed as the weighted means from
123 p.m. and a.m. milk yields on each test day. Blood samples were collected on the last day of wk 5 at
124 0500, 0700 and 1100 h, respectively. Ten mL of blood samples were collected every point-in-time from
125 the coccygeal vein into Vacutainer tubes which included anticoagulation (heparin sodium). After
126 sampling, tubes were kept on ice and immediately transported to the laboratory for centrifugation at
127 4000 × g for 10 min at 4ºC, and plasma was stored at -80ºC until assayed.

1.4 Chemical analyses

The DM and CP of *in vitro* fermentation substrates, concentrates, forage, orts, DGS and beet pulp were analyzed using the procedures of the Association of Official Analytical Chemists (AOAC, 2002). The NDF and ADF contents of the samples were determined using a Fibretherm Fiber Analyzer (Gerhardt, Bonn, Germany) according to Van Soest et al. (1991) with addition of sodium sulphite and alpha-amylase in the NDF analysis. The filtered residues were dried at 105ºC for 12 h and weighed for *in vitro* DM disappearance (**IVDMD**) determination. The NDF contents of the dried residues were determined to calculate *in vitro* NDF (**IVNDFD)**. Total groos energy (**TE**) content was determined by an isothermal automatic calorimeter (5E-AC8018, Changsha Kaiyuan Instruments Co., Ltd, China)

The NH_3-N and VFA concentration was determined according to Chen et al (2017),

Milk samples were analyzed for fat, protein, lactose, SNF and TS by infrared methods (Foss North America, Eden Prairie, MN; Ag-Source, Verona, WI).Glutamic-pyruvic transaminase (**GPT**), plasma ammonia (**AMM**), amylase (**AMY**), cholesterol (**CHO**), glucose (**GLU**), lactate dehydrogenase (**LDH**), triglyceride (**TG**), total protein (**TP**), and urea nitrogen (**UN**) were analyzed by kits (Beijing Leadman Biochemical Co., Ltd, Beijing, China) using auto-biochemical analyzer (Beckman CX4, Beckman Coulter, Inc. USA).

Calculation and Statistical Analysis

During the initial stages of the *in vitro* experiment, the correlation between the pressure in fermentation bottles and gas volumes was measured at 39ºC, and the regression equation was then established:

$$y = 1.506x \ (n = 20, R^2 = 0.999, P < 0.0001) \quad (1)$$

Where y represents gas volume (ml), x is the pressure in bottle (kPa), and 1.506 is a constant. The measured pressure was then converted to gas production (ml). *In vitro* gas production (GP) at 0, 1, 2, 4,

150 6, 12, 24, and 48 h were fitted to a logistic-exponential equation (Wang et al. 2011):

151 $$GP=V_f*(1-\exp(d-t*k))/(1+\exp(b-k*t)) \quad (2)$$

152 Where *GP* represents gas production at *t* time, *Vf* is the maximum gas production (ml), *k* represents gas

153 production fraction (/h), *b* and *d* represent the shape of the gas production curve. The time ($t_{0.5}$, h) when

154 half of the maximum gas production was achieved and the initial fractional rate of degradation (FRD_0,

155 /h) were respectively calculated by employing the following two equations (Wang et al. 2011; Wang et

156 al. 2013):

157 $$T_{0.5}=\ln(\exp(b)+2\exp(d))/k \quad (3)$$

158 $$FRD_0=k/(1+\exp(b)) \quad (4)$$

159 The GP, IVDMD and IVNDFD were corrected by subtracting the values obtained for the blanks. Data

160 were analyzed by two-way ANOVA using the MIXED procedure of SAS (2001), and the incubation

161 time was treat as a repeated factor. Results of milk production, milk quality and blood parameters were

162 statistically analyzed using ANOVA and the MIXED procedure of SAS (2001). Duncan's multiple

163 range tests were used to compare differences among the three treatments. A P-value < 0.05 indicated

164 statistical significance.

165 **2 Results**

166 *2.1 In vitro experiment*

167 2.1.1 In vitro gas production characteristics of different molasses-adsorbents

168 The maximum gas production (V_f) and $t_{0.5}$ of SH were both greater ($P < 0.01$) than that of CRP, WB,

169 RH and DB, while no significant differences ($P > 0.05$) were observed among the other four

170 molasses-adsorbents (Table 3). However, the FRD_0 (0.022 $mL \cdot h^{-1}$) of SH was the least among all

171 molasses-adsorbents, and it was less than that of WB, RH and DB.

2.1.2 IVDMD, and IVNDFD of different molasses-adsorbents

Differences ($P < 0.0001$) in IVDMD among the five molasses-adsorbents were observed (Table 3), with the IVDMD observed for WB (69.82%) being 27.3, 34.5, 3.0 and 17.3% greater than that of CRP, RH, DB, and SH, respectively. The IVNDFD of WB and SH were greater ($P < 0.0001$) than that of other three molasses-adsorbents, with the lowest IVNDFD observed for RH (4.32%).

2.1.3 pH and NH_3-N concentration of *in vitro* incubation fluids for different molasses-adsorbents

The range of pH values of the *in vitro* fermentation fluids was 5.89 to 6.75. The lowest pH value was for WB, with it being less ($P < 0.0001$) than that of the other four molasses-adsorbents (Table 4). The greatest NH_3-N concentration (35.2 mg/dL) was obtained for WB, with it being greater ($P < 0.0001$) than that of the other four molasses-adsorbents.

2.1.4 VFA content of in vitro incubation fluids for different molasses-adsorbents

Acetate content of SH was greater ($P < 0.0001$) than that of the other four molasses-adsorbents (Table 4). The propionate content of SH was the greatest among the five molasses-adsorbents, with it being greater than ($P < 0.01$) that of CRP and RH. Butyrate content of WB was 56.1, 28.7, 18.8 and 20.7% greater ($P < 0.0001$) than that of CRP, RH, DB and SH, respectively. The SH and WB also had the greatest content of TVFA ($P < 0.0001$). There were no differences in A:P ($P > 0.05$) for all five molasses-adsorbents.

2.2 In vivo experiment

2.2.1 Milk performance

The milk yield was 25.0 and 17.0 kg during early- and mid-lactation, respectively, and there were no differences ($P > 0.05$) among the three treatments for either lactation period (Table 5). The contents of lactose and SNF were not affected ($P > 0.05$) by the replacement of molasses-adsorbents. The

194 milk fat and total solids contents of cows fed the WB treatment were greater ($P < 0.01$) than those fed
195 control and SH treatments in early lactation, while there were no treatment differences ($P > 0.05$) in
196 mid-lactation. The milk protein content of cows fed the CG treatment decreased by 0.34%, 0.20% and
197 0.17%, 0.16% ($P < 0.01$) compared with that of WB and SH treatments in early- and mid-lactation
198 periods, respectively.

199 2.2.2 Blood biochemistry indexes

200 The plasma GPT concentration of control was less ($P < 0.01$) than that of WB and SH treatments in
201 early- and mid-lactation periods, while there was no differences ($P > 0.05$) between WB and SH
202 treatments (Table 6). Plasma TP concentration of control was greater ($P < 0.01$) than that of WB and
203 SH treatments in mid-lactation period. The AMY concentration of WB treatment was 96.64% and
204 32.50% greater ($P < 0.01$) than that of control in early- and mid-lactation periods, respectively, while
205 there was no difference ($P > 0.05$) in AMY concentration between WB and SH treatments. The
206 plasma LDH concentration of WB treatment was 20.87% greater ($P < 0.01$) than that of CG treatments
207 in the mid-lactation period. No differences ($P > 0.05$) in plasma AMM, CHO, GLU, TG and UN
208 concentration were found among three treatments in both early- and mid-lactation periods.

209 **3 Discussion**

210 *3.1 In vitro gas production characteristics of different molasses-adsorbents*

211 *In vitro* maximum gas production is an important parameter to evaluate rumen fermentation in
212 ruminants because it provides valuable information about the kinetics of feed digestion in the rumen
213 and reflects the utilization efficiency of fermentation substrates (Metzler-Zebeli et al., 2012). Khazaal
214 et al. (1993) reported that maximum gas production was positively related to hemicellulose and crude
215 protein (CP) contents, while other studies observed a negative relationship between gas production and

CP content of fermentation substrates *in vitro* (Cone and van Gelder, 1999; Tolera and Sundstol, 1999). The current results showed that V_f of SH was greater than that of other the four soybean molasses-adsorbents, due to their differing chemical composition, especially the ratio of non-structural carbohydrate to CP which plays an important role in *in vitro* gas production (Tang et al., 2006). Indexes of FRD_0 and $t_{0.5}$ usually reflect the rate of degradation at an early incubation stage of < 12 h and the incubation time of reaching half of the maximum gas production, respectively. Generally speaking, the faster FRD_0 is, the shorter $t_{0.5}$ becomes (Wang et al., 2013). In the present study, FRD_0 of SH was the least while $t_{0.5}$ of SH was greatest. These variations of FRD_0 and $t_{0.5}$ should be ascribed to differences of nutrients content among the five soybean molasses-adsorbents.

3.2 IVDMD, and IVNDFD of different molasses-adsorbents

In vitro DM disappearance can reflect the extent of fermentation of substrates by ruminal microorganisms. Our results showed that IVDMD and IVNDFD of WB were the greatest among the five soybean molasses-adsorbents. It has been shown that dietary molasses supplementation can improve nutrient digestibility in lactating cows, particularly for fiber (Broderick and Radloff, 2004). Usually, dietary sugars undergo rapid fermentation in the rumen of dairy cows, theoretically leading to lactic acid production and decline of ruminal pH, which potentially depresses fiber digestibility (Oelker et al., 2009). However, Broderick and Radloff (2004) reported that replacing high-moisture corn with molasses improved fiber digestibility, likely reflecting a stimulatory effect of molasses on fiber-digesting ruminal bacteria. In the present study, although the adsorbed concentration of soybean molasses was the same, the chemical composition of the molasses-adsorbents differed due to the substrate itself and possibly due to interaction between soybean molasses and the substrate. The rumen is a very complex ecosystem in which numerous microorganisms and factors play an important role in

238 nutrient degradation. Further study is thereby needed to investigate the mechanism of soybean molasses
239 supplementation on the activity of ruminal amylolytic, proteolytic and cellulolytic bacteria during the
240 processes of *in vitro* fermentation.

241 *3.3 In vitro fermentation parameters of different molasses-adsorbents*

242 As pH value is an important index reflecting the internal homeostasis of the rumen environment,
243 maintaining a relatively stable ruminal pH is vital to assuring efficient rumen fermentation.
244 Ruminants usually possess highly developed systems to maintain ruminal pH value within a
245 physiological range of about 5.5-7.0 (Krause and Oetzel 2006). In the present study, the pH of *in vitro*
246 incubation fluids ranged from 5.89 to 6.75 for the five soybean molasses-adsorbents. Thus, the highly
247 buffered system maintained suitable conditions for fermentation, microbial growth, and fiber
248 degradation in the rumen (Stewart et al., 1997). Sari et al. (2015) reported that low ruminal pH
249 decreased NH_3-N concentration and increased non-ammonia N flow compared with high ruminal pH in
250 beef cattle fed diets containing barley straw or non-forage fiber sources. Khalili (1993) found that
251 molasses supplementation linearly decreased the mean value of rumen pH from 6.6 to 6.2 with the
252 increasing levels of molasses fed to crossbred non-lactating cows. However, in our study there was no
253 consistency between NH_3-N concentration and pH in *in vitro* fermentation fluids, likely because the
254 batch culture system was highly buffered. The inconsistency between in vivo and in vitro results may
255 relate to the buffering capacity of the two systems.

256 Simultaneously, ruminal NH_3-N concentration reflects the equilibrium state for CP degradation and
257 synthesis under specific dietary conditions. As an important nitrogen source for microbial growth and
258 protein synthesis, ruminal NH_3-N has a low efficiency for milk protein synthesis partially due to
259 NH_3-N losses in the rumen (Tamminga, 1992; Hristov and Ropp, 2003). Satter and Slyter (1974)

suggested that the NH_3-N concentration of rumen fluid should not be less than 5 mg/dL to maintain a high growth rate of bacteria. Deficiency of NH_3-N restricts microbial protein synthesis, while high NH_3-N concentration inhibits the microbial NH_3-N utilization in the rumen (Hristov et al., 2002). In our study, the NH_3-N concentrations in *in vitro* fermentation fluids for all five molasses-adsorbents exceeded 5 mg/dL, indicating that the molasses-adsorbents did not restrict ruminal bacterial growth and microbial protein synthesis during the fermentation processes. Meanwhile, feeding a sugar-based product within a diet can change ruminal fermentation pattern, and then further change ruminal NH_3-N concentration (Broderick and Radloff, 2004; DeFrain et al., 2006). In the present study, the different CP contents of the five molasses-adsorbents probably caused the differences of NH_3-N concentration in *in vitro* fermentation fluids. In a number of studies, researchers have also observed strong correlation between dietary CP content and NH_3-N concentration (Broderick and Clayton, 1997). Additionally, the difference in amount and activity of the protein-decomposing microbes for the five substrates might have led to the difference in NH_3-N concentration. Many studies have demonstrated that protein-decomposing microbes (e.g., *Prevotella sp.*) play an important role in the degradation of CP to NH_3-N (Jouany, 1996; Wallace, 1996).

3.4 In vitro VFA content of different molasses-adsorbents

Ruminal volatile fatty acids (VFAs) are major energy sources for ruminants and differences in the total and proportions of individual VFA are important physiological indices that reflect rumen digestion and metabolism. Ruminal microorganisms can transform carbohydrates (e.g. fiber, starch and soluble sugar) to pyruvic acid, which can be further transferred into different VFAs by metabolic pathways. Several studies have confirmed that molasses addition can reduce the ruminal acetate concentration but increase the ruminal butyrate and propionate concentration *in vitro* and *in vivo* (Hristov and Ropp, 2003;

282 DeFrain et al., 2006; Ferraro et al. 2009). However, Martel et al. (2011) reported that dietary molasses
283 supplementation increased the molar proportions of acetate and butyrate, but decreased the proportions
284 of propionate and total VFA (TVFA) in the rumen of dairy cows. Broderick and Radloff (2004)
285 proposed that increased sugar intake (as dried molasses) does not alter the ruminal concentration of
286 total VFA, acetate, butyrate, or any other individual VFA. In the present study, the concentration of
287 acetate, propionate, butyrate and TVFA of *in vitro* incubation fluids were significantly different for the
288 five soybean molasses-adsorbents, and the largest value of TVFA was obtained for SH, implying that
289 SH may provide more energy for ruminants. Moreover, the variations in VFA concentration might be
290 associated with the differences in ruminal OM digestibility of five molasses-adsorbents (Calsamiglia et
291 al., 2008). Comprehensively considering the *in vitro* fermentation characteristics of the five
292 molasses-adsorbents, especially *in vitro* disappearance of NDF and VFA concentration, two
293 molasses-adsorbents (i.e., wheat bran-molasses, WB; soybean hull-molasses, SH) were selected for
294 further in vivo experiment.

295 *3.5 Milk performance*

296 Dietary sugar supplementation can be beneficial for stimulating ruminal microbial protein formation
297 from rumen degradable protein; thus, yield of milk and particularly milk protein content can be easily
298 affected by sugar feeding (Broderick and Radloff, 2004). In the present study, differences in milk
299 production were not observed in early- and mid-lactation, while the milk fat content in early-lactation
300 was improved when dietary corn (100 g/kg) and wheat bran (50 g/kg) were replaced with SH at 150
301 g/kg of dietary DM, or dietary wheat bran was replaced with 150 g/kg of WB. This result is similar to
302 the previous findings of Martal et al. (2011), who reported that dietary molasses supplementation
303 increased milk fat concentration without significantly affecting milk yield when molasses replaced corn

304 at 50 g/kg dietary DM. However, Brito et al. (2014) reported that yields of milk and milk components
305 can be decreased in lactating cows fed flaxseed meal-based diets supplemented with molasses (liquid
306 molasses plus flaxseed meal vs corn meal plus flaxseed meal). This difference might result from the
307 different molasses sources and dietary composition.
308 Yan et al. (1997) demonstrated that when molasses inclusion in the diets fed to mid-lactation cows
309 increased from 156 to 468 g/kg DM, milk protein concentration increased from 31.6 to 33.6 g/kg.
310 Keady and Murphy (1998) observed that supplementing sucrose (10 g/kg DM) significantly increased
311 milk protein concentration of lactating dairy cows. The above-mentioned findings support our results
312 that milk protein content was both significantly increased when WB replaced corn at 150 g/kg DM and
313 SH replaced corn at 50 g/kg DM and wheat bran at 100 g/kg DM in early- and mid-lactation periods.
314 Furthermore, Murphy (1999) concluded that milk protein yield can increase when dairy cows are fed
315 rumen-fermentable energy in the form of molasses in a grass silage-based diet. It was suggested that
316 ruminal microbial protein synthesis can be stimulated and a greater proportion of degradable N can be
317 captured by rumen microbes for dairy cows, leading to increased milk protein synthesis. Therefore, the
318 increment of milk protein content in early- and mid-lactation periods is consistent with the previous
319 literature (Broderick et al., 2004).
320 *3.6 Blood metabolites*
321 The greater plasma GPT concentration observed when SH replaced corn at 150 g/kg DM and WB
322 replaced corn at 50 g/kg DM and wheat bran at 100 g/kg DM in the dietd fed to cows in early- and
323 mid-lactation might be due to more production of alcohol in the rumen for SH and WB treatments.
324 Generally, *Saccharomyces cerevisiae* populations in the rumen increase when molasses is added to
325 dairy rations, and more alcohol may have been produced by *Saccharomyces cerevisiae* during

326 fermentation of SH and WB treatments. Once in the liver, alcohol casues a rise in plasma GPT
327 concentration (Li et al., 2012; Han et al., 2017) .
328 Dairy cows with high genetic merit require an energy-dense diet to fulfill their production potential,
329 and thus starchy cereals are prevalent in the diets of high-producing dairy cows (Nozière et al., 2014).
330 In the present study, the greater plasma AMY concentration of WB treatment in early- and
331 mid-lactation compared with that of the control treatment probably resulted from molasses being
332 fermented rapidly in the rumen supplying energy to the ruminal microbes leading to a more efficient
333 utilization of starch.
334 Some studies have shown that LDH can be affected many factors in dairy cows (Chagunda et al., 2006;
335 Piccinini et al., 2007; Wenz et al., 2010). Nyman et al. (2014) confirmed the hypothesis that LDH can
336 indicate inflammation. In the present study, plasma LDH concentration for WB treatment was greater
337 than that of control treatment in mid-lactation. These probably because of the higher formation of lactic
338 acid and lower rumen pH, it resulted in rumen acidosis and caused inflammation.
339 Lesmeister and Heinrichs (2005) reported no significant differences in blood TP concentration between
340 calves receiving starters containing molasses at 50 and 120 g/kg DM. Azizi-Shotorkhoft et al. (2013)
341 also found no significant difference in blood TP concentration for Moghani sheep fed different levels
342 of molasses (0-100 g/kg). In the current study, the significant changes in plasma TP concentration
343 observed among treatments in mid-lactation, the reasons probably ascrible to the difference in
344 ingredients of experiment diets among treatments. No significant change were observed in
345 early-lactation likely due to the relatively small DMI and a short feeding of the dairy cows during the
346 early lactation period, and the significant differences among treatments during mid-lactation period
347 would be probably resulted from the accumulative effect for a longer feeding.

Thomas et al. (1988) proposed that plasma UN concentration reflects dietary protein intake. Rusche et al. (1993) reported that feeding sources of CP that are less degradable in the rumen decreases blood UN concentration. The lack of significant difference in plasma UN concentration in the present study was in agreement with the findings of Hatfield et al. (1998), who found that molasses type had no effect on plasma UN in sheep.

4 Conclusion

Two molasses-adsorbents (soybean molasses adsorbed by wheat bran and soybean hulls) improved maximum gas production, ruminal total VFA content and NDF degradation *in vitro*. Replacement of dietary corn meal/wheat bran by soybean molasses-adsorbents in the diet of dairy cows increased milk protein and fat contents. We conclude dietary corn meal/wheat bran replacement by soybean molasses-adsorbents promotes ruminal fermentation and improves milk quality in lactating dairy cows.

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514 stress. *Chinese Journal of Animal Nutrition*, 25, 163-170.

515 **Table 1.** Chemical composition of five soybean molasses adsorbed substrates

Items ‡	Substrates †				
	CRP	WB	RH	DB	SH
DM (%)	88.82	91.27	92.61	88.99	88.38
N (%)	1.25	3.17	1.34	3.04	2.12
TE（Mcal/g）	17.91	18.17	16.70	16.64	17.34
NDF (%)	56.47	29.87	54.61	23.68	52.85
ADF (%)	35.30	9.94	45.44	9.44	39.59

516 † CRP = corncob powder-soybean molasses adsorbent; WB = wheat bran-soybean molasses adsorbent;

517 RH = rice husk-soybean molasses adsorbent; DB = defatted bran-soybean molasses adsorbent; SH =

518 soybean hulls-soybean molasses adsorbent.

519 ‡ DM = dry matter; N = nitrogen; TE = total energy; NDF = neutral detergent fiber; ADF = acid

520 detergent fiber.

521 **Table 2.** Ingredients and chemical composition of experimental diets

Ingredient	Group		
	CG	SH †	WB ‡
	Feeding (kg/d • cow)		
Rice straw	6.0	6.0	6.0
Beet pulp	3.0	3.0	3.0
DGS[3]	3.0	3.0	3.0
Concentrate	4.0	4.0	4.0
	(% of DM)		
Corn meal	43.1	33.1	43.1
Soybean meal	10.0	10.0	10.0
Wheat bran	18.0	13.0	3.0
DGS§	21.0	21.0	21.0
SH adsorbed molasses	-	15.0	-
WB adsorbed molasses	-	-	15.0
$CaHPO_4$	1.5	1.5	1.5
$CaCO_3$	1.3	1.3	1.3
$NaHCO_3$	0.6	0.6	0.6
NaCl	0.5	0.5	0.5
Premix¶	4.0	4.0	4.0
Chemical composition of concentrate (% of concentrate DM)			
Dry matter	93.98	94.11	94.63
Crude protein	18.89	19.66	19.13
Calcium	1.90	1.86	1.89
Phosphorus	0.80	0.77	0.79
Neutral detergent fiber	18.70	17.22	18.03
RDP, % of CP	59.81	60.14	59.29

522 † SH = soybean hulls-soybean molasses adsorbents.

523 ‡WB = wheat bran-soybean molasses adsorbents.

524 §DGS = distillers grains with solubles.

525 ¶ Premix（ /kg） ： 113.85g $MgSO_4•H_2O$, 2.69 g $FeSO_4•7H_2O$, 2.55 g $CuSO_4•5H_2O$, 9.54 g

526 $MnSO_4 \cdot H_2O$, 9.60 g $ZnSO_4 \cdot H_2O$, 30 mg Na_2SeO_3, 60 mg KI, 180 mg $CoCl_2 \cdot 6H_2O$, 500,000 IU

527 Vitamin A, 60 kIU Vitamin D, 2000 IU Vitamin E.

528 **Table 3.** Effects of different soybean molasses adsorbed substrates on *in vitro* gas production
529 parameters, IVDMD and IVNDFD

Items[§]	Substrates [†]					SEM [‡]	*P*
	CRP	WB	RH	DB	SH		
V_f (mL)	169.7^{b}	189.1^{b}	158.5^{b}	171.2^{b}	323.4^{a}	27.01	**
FRD_0 (mL·h^{-1}) (10^{-2})	3.89^{b}	6.42^{a}	6.59^{a}	6.79^{a}	2.20^{b}	0.790	**
$T_{0.5}$ (h)	16.71^{b}	10.95^{b}	10.5^{b}	10.09^{b}	31.37^{a}	2.160	***
IVDMD (%)	42.50^{d}	69.82^{a}	35.36^{e}	66.81^{b}	52.56^{c}	0.39	***
IVNDFD (%)	17.41^{c}	34.42^{a}	4.32^{d}	24.89^{b}	33.71^{a}	0.89	***

530 [†] CRP = corncob powder-soybean molasses adsorbent; WB = wheat bran-soybean molasses adsorbent,
531 RH = rice husk-soybean molasses adsorbent; DB = defat bran-soybean molasses adsorbent, SH =
532 soybean hulls-soybean molasses adsorbent.

533 ‡ SEM was standard error of means.

534 [§] IVDMD = in vitro DM disappearance; IVNDFD = in vitro NDF disappearance.

535 [a, b, c, d. e]Means within a row for different soybean molasses adsorbed fermentation substrates that do not
536 have a common superscript differ ($P < 0.05$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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547 **Table 4.** Effects of different soybean molasses adsorbed substrates on *in vitro* fermentation pH, NH_3-N
548 concentration, and VFAs concentration

Items	Substrates †					SEM ‡	*P*
	CRP	WB	RH	DB	SH		
pH	6.34^{b}	5.89^{d}	6.75^{a}	6.01^{c}	6.00^{c}	0.03	***
NH_3-N (mg/dL)	5.09^{d}	35.18^{a}	15.91^{b}	12.95^{c}	12.68^{c}	0.35	***
Acetate (mmol/L)	32.07^{c}	37.10^{b}	32.98^{bc}	35.73^{bc}	45.29^{a}	1.59	***
Propionate (mmol/L)	19.44^{b}	25.41^{a}	19.83^{b}	24.81^{a}	27.08^{a}	1.45	**
Butyrate (mmol/L)	4.14^{c}	9.42^{a}	6.72^{b}	7.65^{b}	7.47^{b}	0.53	***
TVFA (mmol/L) §	56.65^{c}	75.99^{ab}	61.24^{c}	69.88^{b}	81.99^{a}	2.98	***

549 † CRP = corncob powder-soybean molasses adsorbent; WB = wheat bran-soybean molasses adsorbent;
550 RH = rice husk-soybean molasses adsorbent; DB = defat bran-soybean molasses adsorbent; SH =
551 soybean hulls-soybean molasses adsorbent.

552 ‡ SEM was standard error of means.

553 § TVFA = total VFA.

554 $^{a, b, c, d}$ Means within a row for different soybean molasses adsorbed fermentation substrates that do not
555 have a common superscript differ ($P < 0.05$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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565 **Table 5.** Effect of different soybean molasses adsorbed substrates on milking performance in different
566 lactating period in dairy cows

Items	Group †			SEM ‡	*P* §
	CG	WB	SH		
DMI (kg/d)	14.82	15.02	14.93	0.321	NS
Milk Production (kg/d)					
Early lactation (0-100 d)	24.67	23.05	27.16	0.226	NS
Middle lactation (100-200 d)	17.85	17.61	15.33	0.153	NS
Milk Fat (%)					
Early lactation (0-100 d)	2.96^{b}	3.27^{a}	2.84^{b}	0.093	**
Middle lactation (100-200 d)	3.13	3.05	3.07	0.064	NS
Milk Protein (%)					
Early lactation (0-100 d)	2.51^{b}	2.85^{a}	2.71^{a}	0.054	**
Middle lactation (100-200 d)	2.93^{b}	3.10^{a}	3.09^{a}	0.068	**
Total Solids (%)					
Early lactation (0-100 d)	10.95^{b}	11.63^{a}	11.02^{b}	0.178	**
Middle lactation (100-200 d)	11.65	11.50	11.72	0.119	NS

567 † CG was the control group without supplementation of soybean molasses; WB was the treatments that
568 replaced 15% of corn meal by wheat bran-soybean molasses adsorbent; SH was the treatments that
569 replaced 10% of what bran and 5% corn meal by wheat bran -soybean molasses adsorbent.

570 ‡SEM was standard error of means.

571 § NS means the significance among three experimental treatments was not significant ($P > 0.05$).

572 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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580 **Table 6.** Effects of different soybean molasses-adsorbents on plasma metabolites in different lactating
581 period in dairy cows

Item †	Group ‡			SEM §	*P* ¶
	CG	WB	SH		
GPT (U/L)					
Early Lactation (0-100 d)	14.43^{b}	19.00^{a}	19.16^{a}	0.88	**
Middle Lactation (100-200 d)	14.64^{b}	19.36^{a}	19.00^{a}	1.65	**
AMY (U/L)					
Early Lactation (0-100 d)	72.85^{b}	143.25^{a}	112.00^{ab}	23.46	**
Middle Lactation (100-200 d)	94.64^{b}	125.40^{a}	109.15^{ab}	19.53	**
LDH (U/L)					
Early Lactation (0-100 d)	741.67	771.56	774.50	31.00	NS
Middle Lactation (100-200 d)	702.00^{b}	848.54^{a}	753.93^{ab}	42.61	**
TP (g/L)					
Early Lactation (0-100 d)	74.78	76.38	74.88	10.25	NS
Middle Lactation (100-200 d)	81.08^{a}	75.42^{b}	74.26^{b}	12.11	**

582 † GPT = glutamic-pyruvic transaminase; AMY = amylase; LDH = lactate dehydrogenase; TP = total
583 protein;

584 ‡ CG was the control group without supplementation of soybean molasses; WB was the treatments that
585 replaced 15% of corn meal by wheat bran-soybean molasses adsorbent; SH was the treatments that
586 replaced 10% of what bran and 5% corn meal by wheat bran-soybean molasses adsorbent.

587 § SEM was standard error of means.

588 ¶ NS means the significance among three experimental treatments was not significant ($P > 0.05$).

589 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$