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Original Research

Influence of Dietary Inclusion With Corn and Soybean Oils, in Combination With Live Yeast Culture, on Horse Fecal Methane, Carbon Dioxide and Hydrogen Production

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ABSTRACT

Greenhouse gases mitigation in equine is a new developing area of interest. Methane (CH_4) emission is associated with its negative impacts on the environment and its energy loss processes in foregut and hindgut fermenter where it is produced. Hence, this study was designed to examine the influence of two vegetable oils on carbon dioxide (CO_2), CH_4, and hydrogen (H_2) *in vitro*. Five total mixed rations (TMR) were formulated and used as incubation substrates. Steam rolled corn was replaced by corn oil or soybean oil at 0% (control), 2.4% (low level), and 4.8% (high level) of TMR with 0 and 4 mg of yeast culture per gram dry matter of TMR. The use of yeast and soybean oil resulted in the increased ($P < .05$) pH during fermentation. Inclusion of corn oil without yeast resulted in the highest ($P < .05$) dry matter digestibility, and a high level of corn oil had the highest ($P < .05$) lag time required for CO_2 production, which is one of the contributor greenhouse gases for global warming. However, high soybean oil produced the least fecal CO_2 despite its low lag time. Similarly, inclusion of high soybean oil resulted in the highest ($P < .05$) fecal gas production, whereas high corn oil had the lowest gas production/dry matter degraded. High level of corn oil in equine diet may be used to reduce fecal greenhouse gas emission (44.5, 36.0, and 54.6% for CH_4, CO_2, and H_2, respectively). Low level of corn oil and high level of soybean oil may be used when digestibility is the primary concern, which resulted in 54.9% and 31.1% increase in fecal dry matter digestibility, respectively. Overall, low level of corn oil seems to be having general effectiveness on fermentation in horses.

1. Introduction

Greenhouse gases emissions from livestock are both deleterious and waste of metabolic resources to our environment and feed efficiency, respectively. Methane (CH_4) emissions are associated with its disadvantage on the environment (global warming effect) and its negativity as energy loss processes in foregut and hindgut fermenter where it is produced [1] as well as other H_2, CO_2, N_2, and P, which are greenhouse gases of livestock industry capable of causing global warming and polluting groundwater. Although acetogenesis is the main hydrogen sink in hindgut fermenters, livestock such as pigs, mules, and equine produce CH_4. Johnson and Ward [2] estimated the CH_4 produced in horses to be about 1.2–1.7 Tg. Furthermore, equine contribute about 0.40% (kg CH_4 animal/year) [3]. This quantity is likely to increase as global equine population increases. Because of the varying consequence of climate change on the environment, there could be added negative effect on pasture especially in regions where they have poor distribution of pasture and forage quality.

Presently, global equine population is estimated to be about 58.8 million [4], and 1.33 million horses were in Mexico around 2009 [5] where they are important for agricultural activities [6]. This implies

Animal welfare/ethical statement: The research was performed in accordance with the ethical standard laid down in the 1996 declaration of Helsinki and its later amendments.

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that Mexico horse accounts for at least 2.26% of global equine horse. With the projected global increase in livestock population, continuous impact of global climate change, and expected increase in equine population in México, efforts should be made to combat equine greenhouse gases emission with CH_4 being of particular interest. One of the potent strategies of greenhouse gas mitigations in livestock is dietary manipulation. Animal nutritionists are making effort to improve productivity while reducing the greenhouse gas footprint through either dietary supplementation or anaerobic digestion. This should also be the focus of equine nutritionist where efforts are made to reduce their environmental footprint.

Plant extract, yeast, dietary oil, essential oil, plant seed, and lipid supplementation are options available for CH_4 reduction. The ability of *Saccharomyces cerevisiae* to lower CH_4 is because of the ability to induce acetogens growth [7], which would aim at competing with methanogens for H_2. Oil influences greenhouse output through reduction of protozoa, serving as alternative for hydrogen sink (in the form of biohydrogenation), inhibition of the growth or activities of methanogens, and perhaps reduction of fiber digestibility [8].

Although the impact of plant extracts on foregut and hindgut fermentation parameters during manipulation is attributed to the bioactive compound [8], study has shown that medium chain fatty acids are effective tool in microbial manipulation. As a matter of fact, there are reports of microbial adaptation to plant extract, essential oil, and so on. However, there are limited, if any, on microbial adaptation to medium chain fatty acid especially the C-12:0 and C-14:0. Alternatively, the unsaturated fatty acid in oil could also serve as alternative hydrogen sink thereby reducing hydrogen available for methanogens. These fatty acids have a toxic effect on protozoa and methanogens [9]. Lipid supplementation influences CH_4 reduction based on their fatty acid profile [10], especially the medium-chain fatty acid [11]. Soya bean oil and corn oil are examples of dietary oil that could be used, and they have both demonstrated their ability to reduce CH_4 in ruminant [12,13], which may be attributed to the unsaturated fatty acid, and protozoa reduction (which reduced hydrogen exchange with methanogens). The polyunsaturated fatty acids alter the fatty composition of cell membrane phospholipids of microbes [14] and disruption of the bilayer structure through the double bond shape of the molecules [15]. Other means include alteration in the ratio of acetate to propionate, saturation of the unsaturated fatty acid to saturated fatty acid [1,11,16]—otherwise known as biohydrogenation, which help to reduce hydrogen available for methanogenesis—and toxicity on gut microbes such as bacteria, methanogens, and protozoa [1] and reduces cell wall digestibility especially of fiber breakdown through toxicity to cellulolytic bacteria in roughage-based diet [17].

Little or no studies have evaluated the effect of soya oil, corn oil, and yeast on equine CH_4 production. The objective of the present study was to evaluate the effect of dietary inclusion with two sources of vegetable oils on the greenhouse gases emission as an option of the cleaner environment.

2. Materials and Methods

2.1. Substrate and Yeast Cultures

In the first trial, five total mixed rations (TMR) were formulated, as shown in Table 1, and used as incubation substrates.

Table 1
Diet ingredients and chemical composition.

Diets	Control	Corn Oil		Soybean Oil	
		Low	High	Low	High
Ingredients, %					
Oats	12.0	12.0	12.0	12.0	12.0
Steam rolled corn	25.0	22.6	20.2	22.6	20.2
Steam rolled barley	25.0	25.0	25.0	25.0	25.0
Wheat bran	12.0	12.0	12.0	12.0	12.0
Corn gluten feed	5.0	5.0	5.0	5.0	5.0
Soybean meal	5.0	5.0	5.0	5.0	5.0
Type of oil	0.0	2.4	4.8	2.4	4.8
Molasses	6.0	6.0	6.0	6.0	6.0
Vitamins/minerals	0.1	0.1	0.1	0.1	0.1
Soybean hulls	10.0	10.0	10.0	10.0	10.0
Chemical ration composition, %					
Dry matter	86.45	86.51	86.57	86.51	86.57
Crude fiber	9.43	9.37	9.31	9.37	9.31
Crude protein	13.4	13.18	12.97	13.18	12.97
Digestible energy, Mcal/kg[a]	3.38	3.5	3.63	3.5	3.62
Digestible crude protein, g/kg DM)[b]	98.41	96.75	95.1	96.75	95.1
Ca	1.77	1.85	1.84	1.85	1.84
P	3.54	3.92	3.85	3.92	3.85
Mg	1.56	1.68	1.65	1.68	1.65
Na	0.31	0.32	0.32	0.32	0.32
K	8.46	9.01	8.93	9.01	8.93
Cl	0.68	0.82	0.8	0.82	0.8
Zn	18.54	21.98	21.53	21.98	21.53
Cu	4.73	5.85	5.8	5.85	5.8
Fe	140.4	139.63	138.86	139.63	138.86

Oils Composition, per 100 g[c]	Corn Oil	Soybean Oil
Energy, kcal	900	884
Total fat, g	100	100
Polyunsaturated fatty acids, g	47.7	60.71
Monounsaturated fatty acids, g	33.6	24.28
Saturated fatty acids, g	14.4	15

[a] Digestible energy (Mcal/kg) = (3.6 + 0.211Crude protein + 0.421 Ether extract + 0.015 Crude fibre)/4.184 [21].
[b] Digestible crude protein (g/kg dry matter) =4.49 + 0.8533 Crude protein [22].
[c] Provided by the manufacturer Oléico Coral Internacional, SA de CV, San Luis Potosí, México.

Steam rolled corn was replaced with corn oil or soybean oil (Oléico; Coral Internacional SA de CV, San Luis Potosí, México) at 0% (control), 2.4% (low level), and 4.8% (high level) of TMR. Yeast culture (Procreatin 7; Safmex/Fermex SA de CV, Toluca, Mexico) was used in powdered form, and it contained 1×10^{10} cell/g and added to each TMR at 0 and 4 mg/g dry matter (DM).

2.2. In Vitro Incubations

Before incubation begins, fecal contents (the inoculum source) were collected directly from the rectum of six adult English Thoroughbred horses at the Hospital de Grandes Especies de la Facultad de Medicina Veternaria y Zootecnia, Universidad Autónoma del Estado de México, Mexico (aged 9–11 year and weighing 510 ± 20 kg) before the morning feeding. Horses were daily fed 1.5 kg twice a day of commercial concentrate (HORSE POWER Alimento Rolado para Caballo, ALPLA Mexico) and oat hay *ad libitum*. Fecal contents from individual horses were mixed to obtain a homogenized sample of feces of each treatment and mixed with the Goering and Van Soest [18] buffer solution without trypticase in a ratio of 1:4 weight/volume. The incubation media after been mixed were strained through four layers of cheesecloth into a flask with an O_2-free headspace and used to inoculate three identical runs of incubation in 120-mL serum bottles containing 0.5 g of DM substrate.

A total of 90 bottles (two yeast doses × three replicates × three runs × five substrates) plus three bottles without substrates and yeast as blanks were used. After filling all bottles, they were flushed with CO_2 and immediately closed with rubber stoppers, shaken, and placed on an incubator set at 39°C. Gas and CO_2 productions were recorded at 2, 4, 6, 8, 10, 12, 24, and 48 hours using the Pressure Transducer Technique (Extech Instruments, Waltham) of Theodorou et al [19]. At the same incubation period, CO_2, CH_4, and H_2 concentrations in the headspace of the bottles were measured using a diffusion-based gas detector (Air Quality Monitor YesAIR; Critical Environment Technologies Canada Inc, Delta, BC, Canada).

At the end of incubation after 48 hours, bottles were uncapped, and pH was measured using a digital pH meter (Conductronic pH15, Puebla, Mexico); the content of each bottle was filtered under vacuum through sintered glass crucibles (coarse porosity no. 1 pore size 100–160 μm; Pyrex, Stone, UK) and incubation residues dried at 105°C overnight to estimate apparent DM degradability (DMD).

2.3. Chemical Analyses and Calculations

Samples of the TMR were analyzed for DM (#934.01), ash (#942.05), N (#954.01), and ether extract (EE; #920.39) according to Association of Official Analytical Chemists [20].

Digestible energy (DE; Mcal/kg) was calculated as DE = (3.6 + 0.211 crude protein [CP] + 0.421 EE + 0.015 crude fiber)/4.184 [21].

Digestible crude protein (DCP; g/kg DM) was calculated as DCP = 4.49 + 0.8533 CP [22].

To estimate the kinetic parameters of gas production (GP), results of GP (mL/g DM) were fitted using the NLIN option of SAS [5] according to the equation of France et al [23] as:

$$A = b \times \left[1 - e^{-c(t-L)}\right]$$

where A is the volume of GP at time t, b is the asymptotic GP (mL/g DM), c is the rate of GP (per hour), and L (hour) is the discrete lag time before GP.

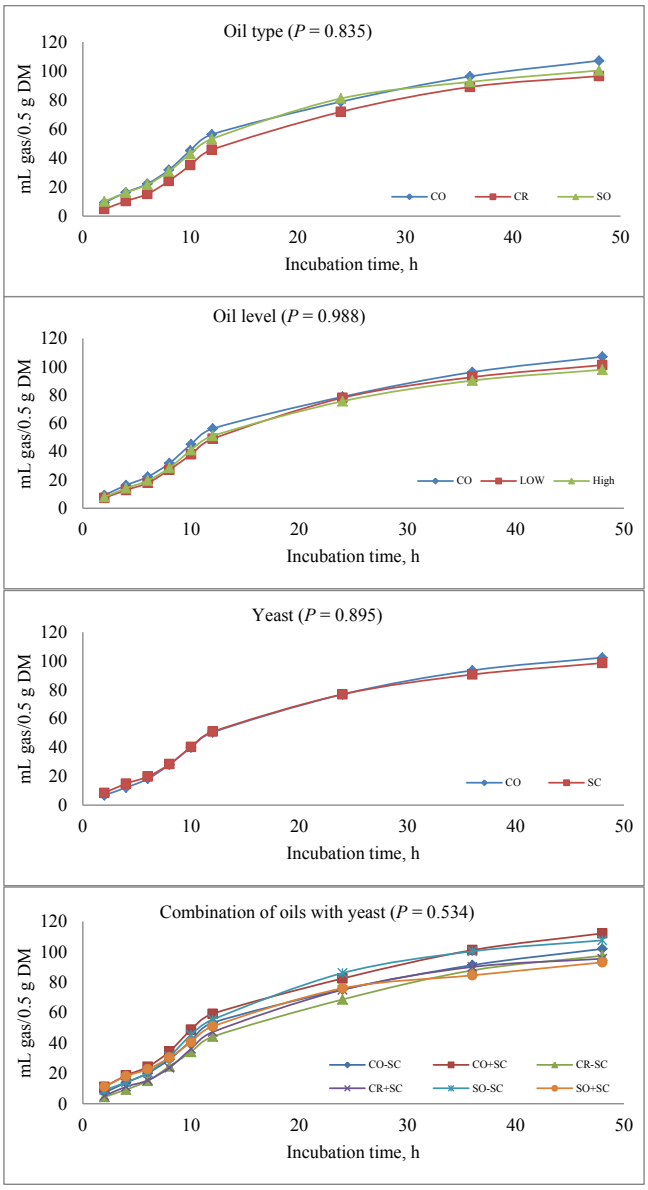

Fig. 1. Horse fecal total gas production (mL/0.5 g DM) at different hours of incubation as affected by the dietary inclusion of corn (CR) and soybean (SO) oils at 0% (CO, control), 2.4% (low), and 4.8% (high) of total diet as well as their combination without (−SC, 0 mg) or with (+SC, 4 mg) live yeast of *Saccharomyces cerevisiae* (SC; per gram dry matter of diet).

2.4. Statistical Analyses

Data of each of the three runs within the same sample of each of the five individual samples of TMRs were averaged before statistical analysis. Mean values of each individual sample were used as the experimental unit. Results of *in vitro* GP and feces fermentation parameters were analyzed as a factorial experiment using the PROC GLM option of SAS [24] as:

$$Y_{ijk} = \mu + R_i + A_j + (R \times A)_{ij} + E_{ijk}$$

where Y_{ijk} is every observation of the ith TMR (R_i) with jth yeast dose (A_j), μ is the general mean, $(R \times A)_{ij}$ is the interaction between ration type and yeast dose, and E_{ijk} is the experimental error. Linear and quadratic polynomial contrasts were used to examine

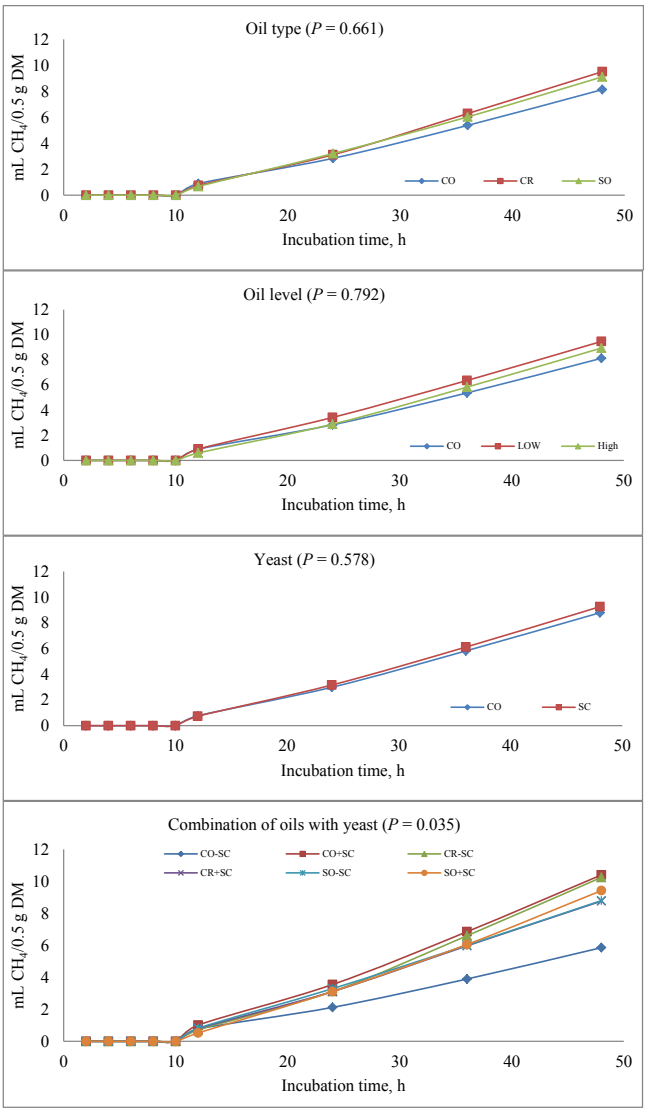

Fig. 2. Horse fecal methane (CH_4) production (mL/0.5 g DM) at different hours of incubation as affected by the dietary inclusion of corn (CR) and soybean (SO) oils at 0% (CO, control), 2.4% (low), and 4.8% (high) of total diet as well as their combination without (−SC, 0 mg) or with (+SC, 4 mg) live yeast of *Saccharomyces cerevisiae* (SC; per gram dry matter of diet).

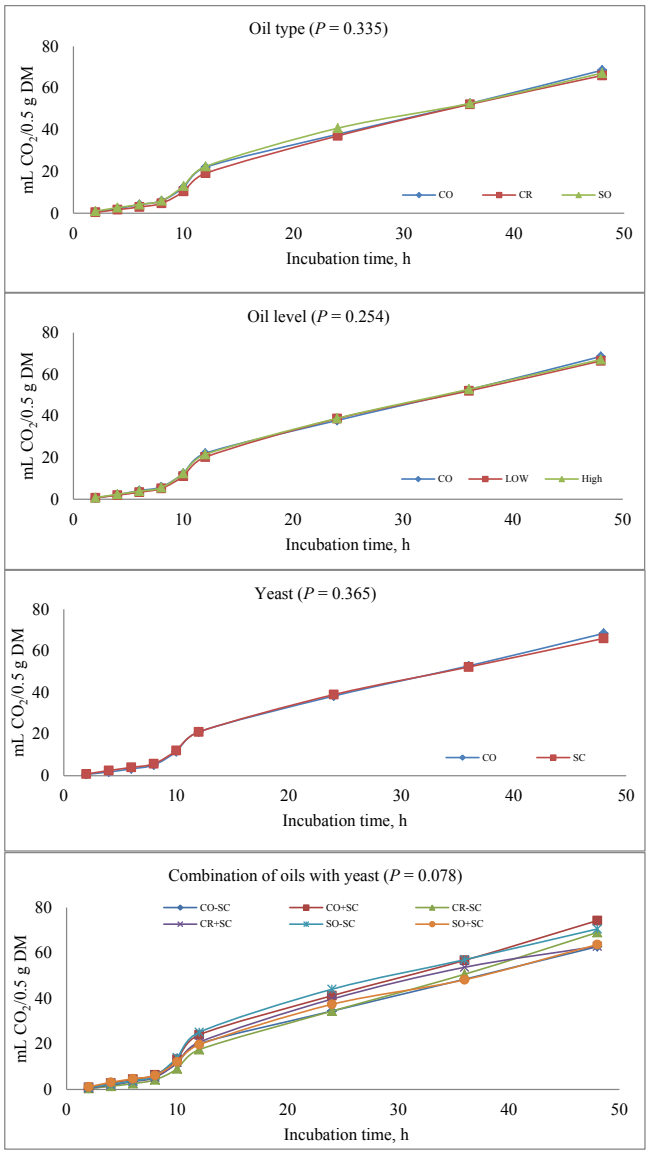

Fig. 3. Horse fecal carbon dioxide (CO_2) production (mL/0.5 g DM) at different hours of incubation as affected by the dietary inclusion of corn (CR) and soybean (SO) oils at 0% (CO, control), 2.4% (low), and 4.8% (high) of total diet as well as their combination without (−SC, 0 mg) or with (+SC, 4 mg) live yeast of *Saccharomyces cerevisiae* (SC; per gram dry matter of diet).

responses to increasing addition levels of test (steam rolled corn replacement and yeast doses). Statistical significance was declared at $P < .05$.

3. Results

3.1. In Vitro Gas Kinetics

Fig. 1 shows that oil type (OT), oil level (OL), yeast, and the interaction between them had no significant effect ($P > .05$) on the asymptomatic GP and the rate of GP. Lag time for asymptomatic GP was influenced ($P = .048$) by OL. There was decrease in lag time required for asymptomatic total GP when high level of oil was used. Treatment had no effect ($P > .05$) on asymptomatic CH_4 GP and lag time (Fig. 2). In addition, asymptomatic production of H_2 and CO_2 was not influenced ($P > .05$) by OT, OL, yeast, and their interaction (Figs. 3 and 4). The interaction of OT × OL influenced ($P = .032$) the lag time of CO_2 production. Furthermore, high level of corn oil resulted in the highest delay, whereas the soybean oil resulted in quick production of CO_2 gas (Fig. 1, Table 2).

3.2. Fecal Fermentation Parameters

The use of OL and *S. cerevisiae* (SC) had impact ($P < .05$) on the pH during fermentation were observed. The inclusion of soybean oil and the presence of SC resulted in increase in the pH level. Interaction of OT × OL ($P = .017$) and OT × SC ($P = .022$) had impact on the DMD. Inclusion of corn oil without yeast resulted in the highest DMD, whereas the lowest ($P < .05$) value was obtained when SC was added to corn oil. Furthermore, the highest DMD was recorded in substrate that had low corn oil inclusion, whereas digestibility decreased ($P < .05$) when high level of corn oil was used.

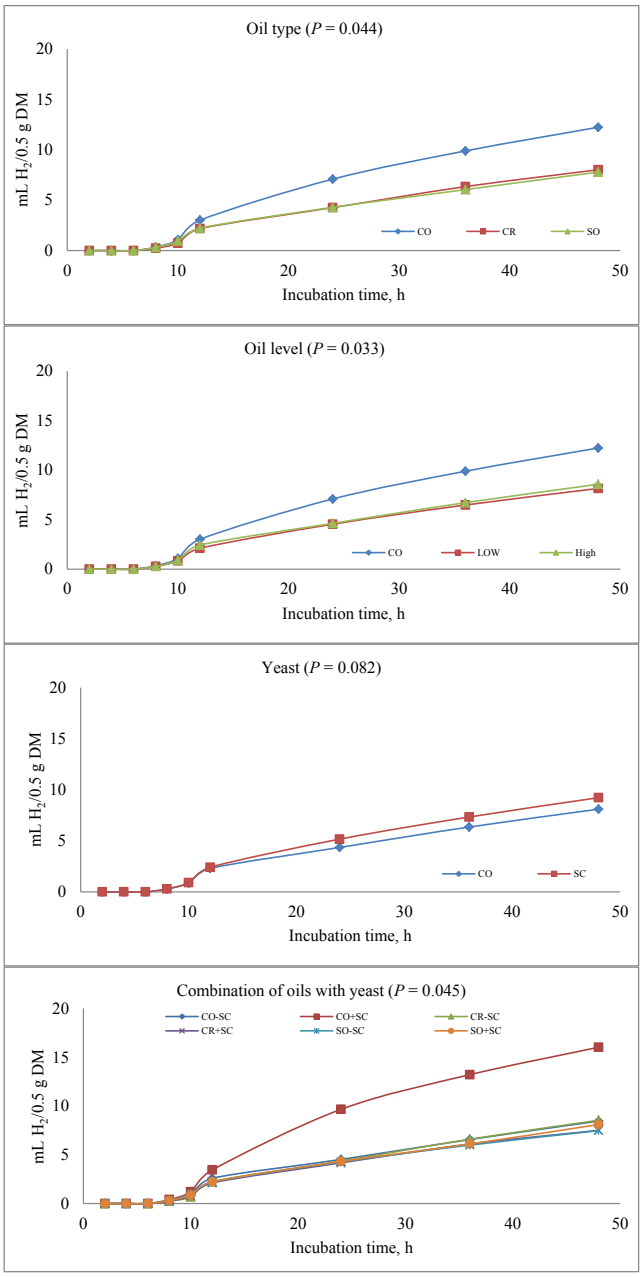

Fig. 4. Horse fecal hydrogen (H_2) production (mL/0.5 g DM) at different hours of incubation as affected by the dietary inclusion of corn (CR) and soybean (SO) oils at 0% (CO, control), 2.4% (low), and 4.8% (high) of total diet as well as their combination without (−SC, 0 mg) or with (+SC, 4 mg) live yeast of *Saccharomyces cerevisiae* (SC; per g dry matter of diet).

However, high soybean oil with SC was an exception because of its high DMD, which was closest to the highest ($P < .05$) DMD in low corn oil without yeast. Conversely, GP production/DM degraded at 8, 12, 24, and 48 hours was influenced ($P < .05$) by OT and their interaction (OT × OL). The OT and OT × OL increased ($P < .05$) the GP/DM degraded at 8, 12, and 24 hours (Table 3).

3.3. Fecal CH_4 Production

The OL × SC had no significant effect ($P > .05$) on CH_4 production/DM incubated. Conversely, low corn oil with the inclusion of SC produced the highest CH_4, whereas high corn oil with the inclusion of SC produced the least CH_4. Still, the lowest value was obtained with OT × OL. The lowest CH_4 value during 24 hours incubation was obtained with the inclusion of high level of corn oil, whereas the highest value was obtained with low corn oil. At 48 hours, the lowest value for CH_4 production/DM degraded was obtained with high level of corn oil, whereas the highest value was with the addition of high soybean oil. The OT, OL, SC, and their interaction had no effect ($P > .05$) on proportional CH_4 production (Table 4).

3.4. Fecal CO_2 Production

There is no effect ($P > .05$) of OL × OT on the CO_2 produced during incubation for 8, 12, 24, and 48 hours. The effect ($P = .039$) of OT was observed in mL CO_2/0.5 g DM degraded at 8 hours of incubation. Corn oil produced the lowest, whereas soybean oil the highest ($P < .05$) value obtained from CO_2/0.5 g DM degraded at 8 hours. The OT × OL showed increasing ($P < .05$) volume of CO_2/DM degraded with time increased from 8 to 24 hours of incubation. However, high corn oil produced the least, whereas high soybean oil produced the highest CO_2 production/DM degraded. In contrast, OT, OL, SC, and their interaction had no significant ($P > .05$) effect on the proportional CO_2 during the 48 hours of *in vitro* fermentation (Table 5).

3.5. Fecal Hydrogen (H_2) Production

The OT, OL, SC, and their interaction had no effect ($P > .05$) on the fecal H_2 production. However, OT had influence ($P = .048$) on H_2 production/DM degraded for 8 hours, but no significant effect at 12, 24, and 48 hours of incubation. Inclusion of corn oil decreased ($P < .05$) the volume of H_2/DM degraded at 8 hours of incubation, whereas H_2/DM incubated increased ($P < .05$) during the 48 hours of fermentation (Table 6).

4. Discussion

Until now, there is not much concern on the need to reduce CH_4 emission from equine. This may be partly due to the fact that the main CH_4 sink in equine is acetogenesis rather than methanogenesis, which made equine emit less CH_4 compared with other herbivores with four-compartment stomach.

Lag time is a measure of the time required for feed digestibility by gut microbes to initiate digestibility. It is important to know that digestibility is an indication of adaptability of microbes to the digesta and the environment, which is an evidence of favorable substrate and environment. The result showed that the high OL created an environment, which is favorable to hence the lower time for, required for GP. This GP indicates that high oil could help animals to have access to nutrient from feed within a short period. Furthermore, high level of corn oil had the highest lag time required for CO_2 production, which is one of the constituents of greenhouse gases for global warming. Contrariwise, high soybean oil had the least delay in producing CO_2. This implies that soybean oil had lower CO_2 inhibiting ability, whereas corn oil could inhibit it better. However, this does not give a better picture. A look at CO_2 showed that although high corn oil had delay in CO_2 production, it actually produced more CO_2 compared with high level of soybean oil, which had the quicker CO_2 production. This means that, on the long term, the microbes in corn oil diet adapted to the oil and produced more gas while the soybean oil inhibited CO_2 despite quick rate of production of gas [25–27]. This points to the inherent ability of soybean oil to reduce CO_2

Table 2
Effect of corn and soybean oils in combination with (+, 4 mg) or without (−, 0 mg) *Saccharomyces cerevisiae* (SC; per gram DM of diet) at low (2.4%) and high (4.8%) levels (percentage of the diet) as feed additives on *in vitro* fecal total gas, CH_4, CO_2, and H_2 kinetics[a] of a total mixed rations fed to horses.

OT	OL	SC	Total Gas			CH_4			CO_2			H_2		
			b	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
Control	0	−	96.6	0.060	2.77	8.6	0.209	15.47	62.8	0.036	6.87	8.23	0.036	9.58
		+	107.3	0.046	2.12	11.4	0.037	17.18	76.7	0.033	7.17	15.51	0.059	10.07
Corn oil	Low	−	88.5	0.065	3.21	10.2	0.044	14.79	66.5	0.036	7.66	6.01	0.038	9.45
		+	103.5	0.053	3.84	14.8	0.037	17.89	70.3	0.039	8.24	9.04	0.039	10.52
	High	−	99.9	0.032	2.26	12.4	0.037	17.53	87.0	0.026	14.88	10.87	0.035	12.30
		+	81.5	0.047	2.38	3.8	0.029	14.02	52.8	0.066	7.99	5.52	0.079	10.45
Soybean oil	Low	−	91.3	0.048	5.02	6.9	0.041	15.43	57.2	0.038	9.53	6.51	0.039	11.20
		+	96.0	0.057	1.14	10.6	0.023	16.78	71.6	0.049	7.34	6.22	0.036	9.37
	High	−	117.0	0.057	1.48	12.2	0.035	17.89	78.1	0.061	6.70	8.05	0.042	7.73
		+	82.5	0.058	1.06	10.2	0.036	18.48	58.3	0.052	6.26	9.64	0.038	9.54
Pooled SEM			20.56	0.0131	1.043	4.16	0.1970	2.154	16.15	0.0132	1.733	6.505	0.0241	1.167
Additive effect[b]														
OT			0.819	0.529	0.317	0.913	0.985	0.479	0.805	0.383	0.076	0.956	0.596	0.147
OL			0.979	0.439	0.048	0.737	0.989	0.623	0.817	0.240	0.534	0.734	0.551	0.875
SC			0.807	0.773	0.155	0.859	0.600	0.512	0.797	0.308	0.120	0.581	0.306	0.993
OT × SC			0.653	0.876	0.094	0.628	0.998	0.702	0.588	0.278	0.457	0.845	0.460	0.821
OT × OL			0.698	0.184	0.684	0.249	0.971	0.391	0.922	0.835	0.032	0.845	0.643	0.074
OL × SC			0.219	0.615	0.325	0.114	0.975	0.234	0.122	0.658	0.251	0.726	0.563	0.829

Abbreviations: DM, dry matter; OL, oil level; OT, oil type; SEM, standard error of the mean.
[a] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (per hour); *Lag* is the initial delay before gas production begins (h).
[b] The *P* value of the interaction among the three experimental factors (OT × OL × SC) was no significant ($P > .5$) for all the measured parameters.

production than corn oil. Hence, as far as greenhouse gas emission in livestock is concerned, the time required for producing this may not be an absolute correct measurement of the impact of the additives, rather, than what quantity were they able to inhibit because initial delay may be because of adaptation rather than inhibition [26–28].

The low pH with inclusion of low corn oil is a further attestation of digestibility, which would have resulted in increased volatile fatty acids production [29]. Conversely, GP/DM degraded showed that OT and OT × OL affected the gas produced within 24 hours. Highest GP was recorded when high level of soybean oil was used, whereas the lowest was gas produced when high corn oil was used. This implied that high level of soybean oil increased digestibility, and it created an enabling microbial environment that favored digestibility [30]. Consequently, high level of soybean oil was not toxic to the microbes, and they were able to adapt to it to favor organic matter digestibility and cell wall carbohydrate digestibility [31].

Neither OT nor OL had influence ($P > .05$) on CH_4 production; However, OT × OL led to the production of lower CH_4. High level of corn oil produced the least CH_4, whereas low level of corn oil produced the highest in 12 and 24 hours of incubation. It may be agreed upon that high level of corn oil is toxic to the methanogens (antimethanogens) and hydrogen-producing

Table 3
Effect of corn and soybean oils in combination with (+, 4 mg) or without (−, 0 mg) *Saccharomyces cerevisiae* (SC; per gram DM of diet) at low (2.4%) and high (4.8%) levels (percentage of the diet) as feed additives on *in vitro* fecal fermentation parameters as well as total gas production at different hours of incubation of a total mixed ration fed to horses.

OT	OL	SC	Fermentation Parameters		Gas Production mL/0.5 DM Incubated				Gas Production mL/0.5 g DM Degraded			
			pH	DMD	8	12	24	48	8	12	24	48
Control	0	−	6.5	19.7	29.3	53.3	75.1	102.0	11.4	21.1	30.2	41.1
		+	6.6	21.1	34.4	59.2	82.5	112.2	14.6	25.0	34.8	46.7
Corn oil	Low	−	6.5	44.2	27.7	48.5	73.9	94.6	10.2	17.5	27.7	35.1
		+	6.6	19.0	24.5	50.6	83.8	109.0	9.7	19.8	32.5	42.0
	High	−	6.5	19.3	21.4	39.9	63.4	100.3	8.1	14.6	22.7	36.7
		+	6.6	14.8	22.8	43.8	66.3	82.0	6.1	12.3	17.4	21.0
Soybean oil	Low	−	6.6	19.5	20.5	38.6	69.9	94.4	8.3	15.7	28.7	38.9
		+	6.7	19.3	30.9	51.3	83.7	100.9	12.4	20.4	33.5	40.4
	High	−	6.6	18.7	41.5	72.3	102.1	120.7	15.4	26.9	37.7	44.9
		+	6.7	34.8	29.9	50.5	68.3	85.3	20.3	34.4	48.0	63.2
Pooled SEM			0.09	6.46	6.63	10.76	17.84	21.66	3.32	5.49	8.98	11.68
Additive effect[a]												
OT			0.045	0.789	0.169	0.333	0.470	0.801	0.023	0.039	0.069	0.119
OL			0.989	0.435	0.529	0.568	0.825	0.865	0.319	0.350	0.892	0.780
SC			0.041	0.561	0.790	0.835	0.929	0.813	0.252	0.273	0.425	0.574
OT × SC			0.925	0.017	0.978	0.621	0.520	0.684	0.233	0.434	0.539	0.394
OT × OL			0.677	0.022	0.145	0.121	0.380	0.604	0.033	0.028	0.094	0.154
OL × SC			0.799	0.051	0.357	0.289	0.286	0.231	0.938	0.905	0.856	0.861

Abbreviations: DM, dry matter; DMD, DM degradability; OL, oil level; OT, oil type; SEM, standard error of the mean.
[a] The *P* value of the interaction among the three experimental factors (OT × OL × SC) was no significant ($P > .5$) for all the measured parameters.

Table 4
Effect of corn and soybean oils in combination with (+, 4 mg) or without (−, 0 mg) *Saccharomyces cerevisiae* (SC; per gram DM of diet) at low (2.4%) and high (4.8%) levels (percentage of the diet) as feed additives on *in vitro* fecal methane (CH_4) production at different hours of incubation[a] of a total mixed ration fed to horses.

OT	OL	SC	mL CH_4/0.5 DM Incubated			mL CH_4/0.5 g DM Degraded			Proportional CH_4 Production		
			12	24	48	12	24	48	12	24	48
Control	0	−	0.77	2.12	5.86	0.30	0.84	2.34	1.35	2.68	5.34
		+	1.02	3.55	10.40	0.45	1.53	4.33	1.78	4.46	9.55
Corn oil	Low	−	1.05	3.61	9.62	0.39	1.46	3.94	2.00	4.10	8.10
		+	0.88	4.40	13.57	0.37	1.71	5.16	1.57	5.23	12.57
	High	−	0.39	2.62	10.87	0.14	0.95	4.08	1.00	4.50	11.50
		+	0.69	1.82	3.96	0.12	0.37	0.92	1.43	2.47	4.53
Soybean oil	Low	−	1.03	2.85	6.37	0.42	1.17	2.65	2.11	4.17	7.97
		+	0.68	3.40	9.66	0.26	1.34	3.79	1.27	3.60	8.27
	High	−	0.61	3.74	11.23	0.24	1.39	4.14	0.90	3.70	9.30
		+	0.37	2.82	9.19	0.20	1.74	5.99	0.73	4.07	10.73
Pooled SEM			0.445	1.066	3.185	0.185	0.466	1.369	0.667	0.901	2.283
Additive effect[b]											
OT			0.800	0.909	0.861	0.820	0.395	0.527	0.603	0.768	0.948
OL			0.219	0.287	0.662	0.171	0.359	0.913	0.137	0.358	0.898
SC			0.971	0.542	0.552	0.973	0.355	0.306	0.887	0.505	0.378
OT × SC			0.569	0.906	0.643	0.772	0.527	0.210	0.596	0.788	0.517
OT × OL			0.913	0.207	0.165	0.617	0.070	0.052	0.751	0.360	0.200
OL × SC			0.645	0.315	0.080	0.835	0.624	0.349	0.421	0.387	0.119

Abbreviations: DM, dry matter; OL, oil level; OT, oil type; SEM, standard error of the mean.
[a] No methane production detected before 12 hr of incubation.
[b] The *P* value of the interaction among the three experimental factors (OT × OL × SC) was no significant ($P > .5$) for all the measured parameters.

microbes or perhaps as reported in [8] medium chain C12:0 and C14:0 are toxic to protozoa. This toxicity to protozoa might have reduced the hydrogen available for CH_4 formation by methanogens [8].

Furthermore, OT × OL–dependent decrease was observed in CO_2 production/DM degraded. High level of corn oil produced the least, which is contrary to the highest produced under the high of soybean oil in 24 hours. The CO_2 produced when high level of soybean oil used was 2.55-fold higher than what was produced by the high corn oil inclusion. On the one hand, this could mean that high corn oil enhances more CO_2 available for volatile fatty acid production thereby making energy more available to equine [32,33]. However, it could be that high corn oil reduced digestibility, hence low CO_2 availability. The second option seems reasonable because data show that high corn oil produced the lowest DMD.

One of the functions of yeast in livestock gut is pH stability; it was observed that yeast had no significant effect ($P > .05$) on the GP, CH_4, CO_2, and H_2. Inclusion of yeast helped to increase the pH during fermentation [26,27,34]. Increase in pH or stability is one of the benefits of yeast in livestock. They help to buffer the internal environment to reduce the impact of acidity by neutralization [35]. This is agreement with the study of Elghandour et al [36] that yeast aids increase in gut pH. The increase in pH could be as a result of the

Table 5
Effect of corn and soybean oils in combination with (+, 4 mg) or without (−, 0 mg) *Saccharomyces cerevisiae* (SC; per g DM of diet) at low (2.4%) and high (4.8%) levels (percentage of the diet) as feed additives on in *vitro* fecal carbon dioxide (CO_2) production at different hours of incubation of a total mixed ration fed to horses.

OT	OL	SC	mL CO_2/0.5 DM Incubated				mL CO_2/0.5 g DM Degraded				Proportional CO_2 Production			
			8	12	24	48	8	12	24	48	8	12	24	48
Control	0	−	5.55	20.09	34.47	62.81	2.15	7.97	13.89	25.35	19.54	37.87	45.94	61.78
		+	6.37	24.04	41.23	74.27	2.66	9.96	17.04	30.30	19.17	40.42	49.23	65.64
Corn oil	Low	−	4.61	20.06	40.38	68.80	1.70	7.33	15.35	25.87	17.33	41.67	53.00	71.00
		+	6.18	21.64	43.47	70.52	2.51	8.60	17.23	27.41	22.67	41.27	51.27	65.23
	High	−	3.89	14.95	28.50	69.30	1.44	5.45	10.21	25.63	17.67	36.67	44.00	71.00
		+	4.40	19.95	36.00	55.38	1.10	5.50	9.39	14.42	19.67	45.67	55.00	68.20
Soybean oil	Low	−	3.69	17.82	35.49	57.93	1.50	7.25	14.49	23.90	18.33	40.67	46.00	57.53
		+	5.72	19.57	36.40	66.35	2.25	7.68	14.41	26.55	19.67	40.67	47.57	65.90
	High	−	8.06	32.53	52.67	83.25	3.05	12.14	19.55	31.26	20.00	43.67	50.67	68.33
		+	6.61	19.86	38.47	61.07	5.01	15.22	30.37	49.49	22.33	39.73	56.07	69.73
Pooled SEM			1.452	5.290	10.570	16.234	0.838	2.877	5.880	9.497	3.826	5.117	6.192	6.984
Additive effect[a]														
OT			0.232	0.384	0.626	0.921	0.039	0.066	0.117	0.167	0.783	0.971	0.866	0.485
OL			0.507	0.587	0.997	0.907	0.272	0.365	0.632	0.529	0.878	0.920	0.655	0.379
SC			0.372	0.890	0.793	0.891	0.126	0.362	0.350	0.508	0.382	0.573	0.260	0.723
OT × SC			0.717	0.250	0.429	0.973	0.349	0.789	0.564	0.262	0.737	0.392	0.896	0.359
OT × OL			0.067	0.153	0.204	0.455	0.016	0.039	0.048	0.114	0.522	0.855	0.299	0.558
OL × SC			0.277	0.467	0.723	0.320	0.980	0.862	0.625	0.917	0.831	0.708	0.350	0.841

Abbreviations: DM, dry matter; OL, oil level; OT, oil type; SEM, standard error of the mean.
Means in the same column with different superscripts differ ($P < .05$).
[a] The *P* value of the interaction among the three experimental factors (OT × OL × SC) was no significant ($P > .5$) for all the measured parameters.

Table 6
Effect of corn and soybean oils in combination with (+, 4 mg) or without (−, 0 mg) *Saccharomyces cerevisiae* (SC; per g DM of diet) at low (2.4%) and high (4.8%) levels (percentage of the diet) as feed additives on *in vitro* fecal hydrogen (H_2) production at different hours of incubation of a total mixed ration fed to horses.

OT	OL	SC	mL H_2/0.5 DM Incubated				mL H_2/0.5 g DM Degraded				Proportional H_2 Production			
			8	12	24	48	8	12	24	48	8	12	24	48
Control	0	−	0.29	2.59	4.52	8.45	0.11	1.00	1.78	3.35	1.00	5.17	6.25	8.42
		+	0.41	3.45	9.66	16.02	0.17	1.44	4.52	7.38	1.17	6.00	11.67	14.92
Corn oil	Low	−	0.28	1.69	3.32	6.15	0.10	0.59	1.22	2.25	1.00	3.67	4.67	6.67
		+	0.25	2.40	4.97	9.22	0.10	0.89	1.84	3.42	1.00	5.00	6.33	9.00
	High	−	0.21	2.78	5.39	10.94	0.08	0.97	1.87	3.85	1.00	6.33	7.67	10.33
		+	0.23	1.84	3.37	5.78	0.06	0.58	0.98	1.60	1.00	4.33	5.33	7.33
Soybean oil	Low	−	0.21	1.63	3.52	6.55	0.08	0.66	1.43	2.68	1.00	3.67	4.67	6.67
		+	0.31	1.83	3.80	6.60	0.12	0.73	1.54	2.66	1.00	3.67	4.67	6.67
	High	−	0.42	2.79	5.01	8.40	0.15	1.01	1.82	3.08	1.00	4.00	5.00	7.00
		+	0.30	2.64	4.82	9.61	0.20	1.98	3.85	8.20	1.00	5.33	7.33	11.33
Pooled SEM			0.090	0.729	4.717	6.908	0.039	0.360	2.614	3.405	0.121	1.317	5.660	6.294
Additive effect[a]														
OT			0.308	0.931	0.994	0.962	0.048	0.188	0.715	0.624	1.000	0.478	0.885	0.932
OL			0.641	0.231	0.825	0.752	0.392	0.108	0.738	0.610	1.000	0.290	0.757	0.720
SC			0.554	0.579	0.569	0.580	0.188	0.132	0.426	0.380	0.458	0.613	0.541	0.494
OT × SC			0.982	0.895	0.972	0.865	0.304	0.269	0.747	0.583	1.000	0.594	0.852	0.798
OT × OL			0.280	0.489	0.879	0.858	0.065	0.138	0.695	0.583	1.000	1.000	0.951	0.878
OL × SC			0.496	0.341	0.757	0.720	0.952	0.841	0.955	0.878	1.000	0.594	0.918	0.959

Abbreviations: DM, dry matter; OL, oil level; OT, oil type; SEM, standard error of the mean.
Means in the same column with different superscripts differ ($P < .05$).
[a] The P value of the interaction among the three experimental factors (OT × OL × SC) was no significant ($P > .5$) for all the measured parameters.

ability of yeast decrease the lactic acid or increase the lactic acid using bacteria [26,37].

Lipid supplementation is a method of manipulating fermentative environment for digestibility and production improvement. One of the main functions of lipid supplementation has been attributed to their fatty acid chain. Medium chain fatty acid and polyunsaturated fatty acids rather than bioactive compound are associated with their function. Furthermore, Marounek et al [22] and Abubakr et al [8] have both attributed the toxicity of the fatty acid to bacteria and protozoa. Corn oil without yeast inclusion led to the highest DMD, an indication of the degree of organic matter breakdown by microorganism and an adaptation to the environment or favorable environment. Corn oil was not toxic to the microbes or microbial adaption. This implies that microbes can readily adapt when the oil is used without being toxic to them compared with soybean oil or high level of corn oil [26]. The increase in DMD also has positive and beneficial implication for volatile fatty acids and energy availability for the animals [27]. Breakdown of cell wall is essential for nutrient assessment, hence the use of low corn oil without yeast or alternatively, and high soybean oil with yeast could be used to increase DMD without disrupting the gut pH and thereby reducing incidence of acidosis.

5. Conclusions

There is no one fit all solution to the problems such as digestibility and greenhouse gas emission in livestock production. To enhance time required for digestion, which is an indication of microbial adaptation, high level of soybean oil may be used to reduce the time required by microbes for digestibility. Inclusion of soybean oil with yeast would help to increase and stabilize gut pH thereby reducing incidence of microbial disruption. Greenhouse gases emission in equine may be reduced by supplementing equine diet with high level of corn oil to reduce the emission of equine by 44.5, 36.0, and 54.6% for CH_4, CO_2 and H_2, respectively. However, for combined performance, such as digestibility, pH, and greenhouse gases emission reduction, soybean oil may be recommended.

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