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Comparative Study on the influence of Three Feed Additives on Methane Production, Rumen Fermentation, and Milk Yield in Dairy Cows

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Abstract | Methane emission from ruminants during the fermentation of feed represents a loss of energy and contribution of greenhouse gas. Feed additives are one of the various dietary strategies that have been used to mitigate and reduce methane production in dairy cows. This aimed to determine the effects of three varieties of feed additives (green seaweed, probiotics *Saccharomyces cerevisiae* or *Yucca schidigera*) on *in-vitro* total gas and methane production, *in-vivo* rumen fermentation parameters, and milk production in dairy cows. Twenty Friesian cows were randomly allocated to four groups (five cows /group). All cows were fed TMR (total mixed rations). The first group was fed TMR without supplementation, groups from 2 to 4 were fed TMR supplemented with one of the feed additives *S. cerevisiae*, yucca, or green seaweed at a rate of 25 g, 20 g, and 100 g/head/day for respectively. The amount of ammonia, short-chain fatty acid, *in-vitro* dry matter digestibility (IVDMD), *in-vitro* organic matter digestibility (IVOMD) were estimated. The results revealed that feed additives enhanced the beneficial processes of the rumen with the reduction in total gas and methane production. All the experimental fed additives had a positive significant effect on the reduction of methane production. Moreover, green seaweed and *yucca* reduced total gas and methane production significantly more than the probiotic *Saccharomyces cerevisiae*. Dietary supplements with probiotics or seaweed recorded a significant increase in short-chain fatty acid, *in-vitro* dry matter digestibility, and *in-vitro* organic matter digestibility than did other groups. However, dietary supplements with *yucca* recorded the lowest value in rumen ammonia concentration and protozoal population. Furthermore, the addition of probiotics or green seaweed to the diet has significantly improved milk yield and composition compared with *yucca* supplementation, as well as decreased the somatic cells count (SCC) as a result of feed additives. Experimental feed additives contributed to enhancing beneficial processes and the reduction of methane production.

Keywords | Gas and methane production, Green seaweed, Milk yield, Probiotics *Saccharomyces cerevisiae*, *Yucca schidigera*

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INTRODUCTION

Greenhouse gas emissions are contributing to a deteriorating environment, an increase in global warming, and causing substantial climate change nowadays. Methane has taken part in global warming about 21 times more than carbon dioxide (Forster et al., 2007). The agriculture fields present a large share of the total methane emissions at approximately 30% from anthropogenic sources, and about 85% coming from the rumen liquor fermentation (Gleik et al., 2010). In ruminants, about 95% of the methane is produced via feed fermentation, and 95 to 99% is exhaled through the nose and mouth; that leads to a loss of about 8-12% of energy depending on the diet ingredients (Haque, 2018).

In Egypt, methane emissions are increasing from 30, 346.4 kt of CO_2 equivalent in 1993 to 51, 976.8 kt of

CO_2 equivalent in 2012, at an average annual rate of 3.92%, according to (WRI CAIT 2.0, 2015). The organic matter of feed is fermented into volatile fatty acids, ammonia, carbon dioxide, and methane. The gases produced during the fermentation represent losing energy that the animal can use to increase production performance. Both methanogenic bacteria and protozoa are associated with methane production in the rumen.

Consequently, a lot of efforts have recently been ongoing to manipulate rumen fermentation and the rumen microbial ecosystem to decrease methane production by one of the following basic principles: Direct inhibition of methanogenesis, decreases the production of hydrogen during fermentation, or alternative pathways for the use of hydrogen (Emilio, 2020; Greening et al., 2019). Dietary strategies are one of the methods to reduce enteric methane emissions. Van Gastelen et al. (2019) reported that any one of these methods such as the level of the feed intake, type of carbohydrate, quality of forage: Concentrate ratios, and the feed additive can affect rumen methane emissions. Thus, the use of any method for methane reduction can only be justified if there is a beneficial effect larger than the cost of the product. Feed additives are products used in animal nutrition to improve the quality of feed and are applied as a modification strategy to improve the animal's performance. They are defined as organic or inorganic substances, micro-organisms, or preparations from plant extracts, which are intentionally added to feed or water. Feed additives that are used to reduce ruminant total gas and methane emissions are ionophores, probiotics, seaweeds, saponins, tannins, organic acids, nitrates, bacteriocins, fats, and essential oils. Most of these additives have a direct effect on rumen manipulation, like inhibition of methanogenesis or enhancing non-methanogenesis by lowering hydrogen production during fermentation (Emilio, 2015). Selecting the feed additives to reduce methane gas is dependent upon many factors, like the type of production (milk or meat), economic benefits, and is safe for the animals. In recent years, many studies have been published to investigate the possibility of reducing methane emissions and reducing the energy losses from methane production. Vrancken et al. (2019), Honan et al. (2021) and Thompson and Rowntree (2020). The main targets are the reduction of methane emissions from ruminants to benefit the lost energy that can be used by the animals to improve their production. Van Gastelen et al. (2015) found that grinding and pelleting of forages and selecting a specific type of feed additive can decrease methane production by more than 30%. Recently, an increase has been observed in the feed market in the number of feed additives, especially probiotics, as well as new types of feed additives such as dried seaweed and *Yucca schidigera*. It has also been noted that these additives, with different compositions and benefits, have the same property of reducing methane emissions from ruminants, especially dairy cows.

This study aimed to determine the effect of green seaweed, probiotics, or *Yucca schidigera* as feed additives on *in-vitro* total gas production and methane production, as well as determine the dry matter and organic matter digestibility and parameters of the rumen liquor fermentation, microflora population, and milk production in dairy cows.

MATERIALS AND METHODS

This study was carried out at Noubaria Station, Animal Production Research Institute, Egypt from November 2020 to May 2021.

Animals, housing, and feeding

This experimental study was approved all procedures involving animals by the researchers committee of Regional Centre for Food and Feed, Agriculture Research Centre, Ministry of Agriculture, Egypt. Approval number: (00031/2020).

This experiment was designed to determine the effects of probiotics (*Saccharomyces cerevisiae)*, *Yucca schidigera*, and dried green seaweeds on *in-vitro* dry matter and organic matter digestibility, methane production, gas production, *in-vivo* liquor rumen fermentation, and productivity of dairy cows. Twenty multiparous lactating crossbred Friesian cows were assigned randomly to four treatments (5 cows/ each treatment) stratified by live body weight (535 ± 7.5 kg).

Housig, the experiment took place from December 2020 to March 2021 at the Noubaria station farm in El-Beheira governorate (46 km south-west of the Alexanderia-CairoDesert Road, at a latitude angle of 30.90670° and a longitude angle of 29.87023°, 30m above sea level). Animals are maintained under an open housing system. Each experimental group was in one large barn oblong, about 18 m in length, 9 m in width, and 4.0 m in height, shaded with corrugated metal sheets. The floor is made from sand and covered with of straw, a urine drainage sub-layer of gravel, Promote drainage by sloping the floor (1 inch per 5 feet) toward an alley channel.Animals exposed to 16 to 18 hours of light obtained partially by artificial light, followed by 6 to 8 hours of darkness. Total individual feeding control is achieved. Each barn contains separate feed bunks. Feed bunks are tilted for the animal's comfort and to provide a convenient environment. Vaccination of animals according to the schedule of vaccinations Calcium chloride It is used as a disinfectant and is used in the form of an aqueous solution of 0.5–2.5% in barns (cows), tool stores. Rodent control procedures and insect control procedures, manure management, remove molasses or brewer's yeast that might accumulate under feeding areas

or in corners, insect light traps eliminate standing water, insecticide applications after cleanup, apply insecticide to various surfaces. The Central Laboratory for Agricultural Climate (CLAC) provided data on ambient temperature and relative humidity from December 2020 to March 2021. The average temperatures were high (17–21 °C) and low (11–15°C), with high (80–66%) and low (55–45%) humidity.

All cows were fed a total mixed ration (TMR) with 58:42% concentrate roughage to meet their nutrient requirements according to NRC (2001) recommendations.

The nutrient contents of feed ingredients and nutrient contents of green seaweeds are shown in Table 1.

The experimental treatment consists of four treatments; the 1st was assigned as a control group and fed total mixed ration (TMR), the 2nd group was fed TMR and supplemented with 25 gm/head/day of commercial probiotic-containing *Saccharomyces cerevisiae* 2.5 × 10^8 (CFU/g) of active yeast cells, the 3rd group was fed TMR which blended with 20 gm/head/day of *Yucca schiagera* (105 g saponins/ kg powder *Yucca schidigera*, and the 4th was fed TMR which blended with 100 gm/head/day of dried green seaweed (*Ulva lactuca*).

Preparation of seaweeds

Seaweed (*Ulva lactuca*) was collected from the coastal line of Alexandria (attached to rocks), rinsed with fresh water, dried at 30°C until a final moisture of 15%, dried green seaweeds grounded through a 1-mm stainless-steel screen using a Wiley mill grinder, dried at 60°C in a forced-air oven for 48 h (AOAC, 2005), and stored for chemical analysis.

Macro and microelements were assessed by Atomic absorption spectrometry contrAA 800.

Total phenolic contents (TPC) in the seaweeds were determined by Folin–Ciocalteu colorimetric method according to Singleton et al. (1999), total flavonoid content of seaweeds were measured using aluminum chloride colorimetric assay after Dewi and Riska (2019), tannins were determined according to Makkar et al. (1993), and Saponin content in *yucca schidigera* was determined gravimetric method described by Harborne (1973).

Table 1: Ingredients and chemical composition of the total mixed ration and green seaweeds.

Item	TMR	Chemical composition of green seaweeds	
Ingredients (g kg^{-1} DM)		DM	85.6
Corn silage (9% CP)	343	Protein%	9.2
DDG***	110	Fiber%	7.1
Corn	102	Fat%	3.7
Wheat grain ground	80	Ash%	20.1
Wheat bran	120	Neutral detergent fibre	22.1
Soybean meal ground (47% CP)	150	Acid detergent fibre	7.8
Calcium carbonate	12	Macro and Micro Minerals	
Rice straw	77	P g/kg	1.4
Vitamin-mineral premix* and salt	6	K g/kg	21.8
Total	1000	Na g/kg	22.3
Chemical composition,(g kg^{-1} DM)		Ca g/kg	18.2
Dry matter	658.1	Fe mg/kg	49
Crude protein	163.1	Zn mg/kg	21.8
Ether extract	29.9	Se mg/kg	12.2
Neutral detergent fibre	395.1	Ar mg/kg	0.47
Acid detergent fibre	244.7	Iodine mg/kg	36.9
Acid detergent lignin	37.9	phytochemical screening	
NFC**	359.2	Total phenol mg/g	9.54
Starch	254.8	Total flavonoids mg/g	2.08
Ash	82.7	Tannins % DM	0.6

*Supplied per kilogram of premix (Kav): Vitamin A 12 000 000 IU, Vitamin D3 3 000 000 IU, Vitamin E 30 mg, Mn 50 mg, Fe 50 mg, Zn 50 mg, Cu 10 mg, I 0.8 mg, Se 0.15 mg, antioxidant 10 mg. **NFC = non fiber carbohydrate (%), calculated as: 100 – [(NDF (%) + CP (%) + EE (%) + ash (%)]. ***DDG: dried distiller grains.

Feed intake, milk sampling, and milk composition

Feed intake was recorded daily by weighing the offered rations and refusals from the previous day. Diets were offered twice a day at 07:00 and 7:00 pm. Samples of TMR were taken daily, dried at 60˚C in a forced-air oven for 48 h (AOAC, 2005) and proximate analysis of the samples for ash, crude protein, fiber, fat, and carbohydrate contents were determined as described by AOAC (2005). Fiber fraction was determined according to Van Soest et al. (1991). The starch contents were assessed after the ICC (2017) generic methods.

Cows were machine milked twice daily at 06:00 am and 6:00 pm from 30 days to 120 days, and samples (100 ml/l of recorded milk yield) were collected at each milking. A mixed sample of milk (proportional to amounts produced in the morning and evening) was taken daily. Milk samples (90 samples/ cow) were analyzed for total solids, fat, protein, and ash after Ling (1963), and lactose was calculated by difference.

Average yields of each milk component were calculated for individual cows by multiplying milk yield by the component content (g/kg) of milk. Fat corrected milk (4 %) was calculated according to Gaines and Davidson (1923) using the following equation:

$$FCM4\% = M\ (0.4+0.15\ F\ \%)$$

Where M= milk yield, F = fat percentage

Milk energy value (E) was calculated after Kleiber (1961):

$$E\ (kcal/kg) = (\%\ fat \times 92) + (\%\ protein \times 58.6) + (\%\ lactose \times 39.5)$$

Energy-corrected milk (ECM) was calculated according to Sjaunja et al. (1991) as:

$$ECM\ (kg/d) = (milk\ production * (383 * \%\ fat + 242 * \%\ protein + 783.2) / 3140)$$

Milk samples for Somatic Cell Count (SCC) the milk samples were heated to 40˚C in a water bath for 15 min. Then the samples were processed in the out counter device according to Gonzalo et al. (1993).

Sampling and analysis of rumen fluid

Ruminal fluid contents were sampled at 0 times before cows feeding and at 3 and 6 hours after the morning feeding using stomach tubing from day 21 to day 24 for the measurements of ruminal fermentation parameters and microbial flora populations. Approximately 200 mL of rumen fluid were collected (27 samples/group), from each treatment (the same cows used in the lactation trials) and strained through 4 layers of cheesecloth. The supernatant was used for determining pH immediately using a pH meter (Orian 2 star digital). Approximately 10 ml of the sample was preserved with 2-3 drops of formalin to prevent fermentation. Ammonia-N (NH_3-N) was determined according to method 973.49 (AOAC, 2005). The concentration of total short-chain fatty acid (TSCFA) was determined according to Anderson and Yang (1992). Concentration and molar proportions of individual SCFA were measured by gas-liquid chromatography. The separation process was carried out with a capillary column and flame ionization detection. The column temperature was adjusted to 100˚C for 1 min, 20˚C/min to 140 ˚C, and 8˚C/min to 200˚C/5 min. Helium was used as the carrier gas.

Rumen protozoa and total bacteria counts were carried out according to Martin et al. (1994). About 50 ml of rumen liquid was filtrated through 2 layers of cheesecloth. The 15-mL aliquot was treated with formalin 1% (wt:v), centrifuged at 500 rpm/5 min, and the supernatant was used after serial dilutions (1:1,000) in saline solution to count total protozoa and bacteria using Sedgewick Rafter counting cell.

The microbial nitrogen (MN) synthesized

Synthesized MN was determined according to Chen and Gomes (1992). The method was based on the measurement of purine derivatives (PD mmol/day) by determining allantoin and uric acid in urine by using a spectrophotometer following Fujihara et al. (1987). Uric acid absorbs at 293 nm, while allantoin at 520 nm. Urine was collected into a container with 100 ml of 10% H2SO4 (0.036 N) to prevent bacterial destruction of PD. Equations were used to calculate microbial nitrogen (MN).

$$MN = (70 \times AP) / (0.83 \times 0.116 \times 1000)$$

Where 70 represents the amount of N in the purines (mg N/mmol), 0.83 is the digestibility of the microbial purines, and 0.116 is the purine N: total N ratio in ruminal microorganisms. The absorbed microbial purines (AP, mmol/day) were calculated from the total excretion of purine derivatives (PD, mmol/day) using the equation following Chen and Gomes (1992).

$$AP = \{PD - (0.385 \times BW^{0.75})\} / 0.85$$

Where 0.85 is the recovery of absorbed purines as urinary purine derivatives, and 0.385* $BW^{0.75}$ is the endogenous contribution in the urinary excretion of PD (Verbic et al., 1990).

Measurement of gas production

In-vitro gas production was determined after Menke and Steingass (1988). Rumen fluid was collected before

feeding in the morning using stomach tubing from cows fed a TMR. Rumen fluid was strained through four layers of gauze into a pre-warmed and insulated bottle. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Samples (200±10 mg) of the oven-dry feedstuffs and the respective mixtures were accurately weighed into 100-ml glass syringes fitted with plungers. *In-vitro* incubation was conducted in one run involving quintuplicate samples. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution after Menke and Steingass (1988). Three blanks containing 30 ml of medium only were included in each assay. The syringes were placed in a rotor inside an incubator (39°C) with about one rotation per min. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72, and 96 hours. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. Gas production was fitted to the non-linear equation model of exponential (EXP0) by Schofield et al. (1994).

$$V = VF\ (1-exp\ (-kt))$$

Where: V, is the cumulative gas production (in ml) at different incubation times; VF, final asymptotic gas volume; {VF= Vfinal - V0 - GP0} where, V final= the final volume of gas recorded at the end of incubation time, V0= the initial volume of gas recorded before incubation starts, GP0 = the mean blank value. *k,* fractional rate of gas production, t, incubation time (h). The fractional rate (μ, h^{-1}). Where μ= the point of inflection of the gas curve at time t.

About 100 ml glass syringes fitted with an extra outlet containing a gas-tight septum for sampling from cumulative gas production. After incubation time at 24, 48, 72, and 96, methane was measured by taking samples of 1 ml from headspace gas from each syringe by evacuated vials and injecting into Gas Chromatography (GC) with flame-ionization detection. (Methane was measured by taking 1 ml from headspace gas from each syringe after incubation time at 24, 48, 72, and 96 hours by evacuated vials and injecting it into gas chromatography (GC) with flame ionisation detection. To collect gas samples from each syringe, the syringe (100 ml) used in trial gas production was equipped with three-way taps (Luer-Lock) and pre-evacuated exetainers).

After the gas was sampled for CH_4 and total gas production was measured. At the end of the fermentation period, the fermented residues were filtered into pre-weighed filter crucibles, dried for 24 h at 105°C, weighed, and *in-vitro* dry matter and organic matter digestibility (IVDMD) that was calculated by a modified Tilley and Terry (1963) technique.

(IVDMD) was calculated after incubation using the following equation:

$$IVDMD\ (\%) = [(1 - \{(residue\ weight\ (DM)\ (sample\ after\ incubation) - Blank\} / sample\ weight)\ (DM) \times 100]$$

$$IVOMD\ (\%) = [(1 - ((residue\ weight\ (OM)\ (sample\ after\ incubation) - Blank) / sample\ weight)\ (OM) \times 100]$$

Blood sampling and analysis

At the end of the trial, blood samples (10 mL) were collected by venipuncture from the jugular veins into plain tubes (red cap BD vacutainer tubes) and allowed to stand at room temperature for 45 min to clot. Samples were centrifuged and the sera were stored at –20°C until analysis. Liver function was assessed by measuring the activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) were measured on a spectrophotometer after Sevinch et al. (2001). Kidney function was evaluated by measuring urea, creatinine, and total protein using a spectrophotometer after (Coles, 1986). Also collected blood by using heparinized vacuum tubes for determining hematology by using hematological analyzer (The scil VET abc, Montpellier, France).

Statistical analysis

Data were subjected to analysis as a completely randomized design with repeated measures using the MIXED procedure of SAS, 2002 (Version 9.2) Statistical processes were carried out using the General Linear. The model describing each trait was assumed to be:

$$Yijkl = \mu + Ti + a\ (T)\ IJ + WK + Eijkl$$

Where;
Yijkl= Parameter under analysis; μ = Overall mean; Ti = The fixed effect of treatment; a (T) IJ = The random effect of animal (j) nested within treatment (i); WK= The fixed effect of week when K = 1, 2,….,8; Eijkl= random error. Significant differences among means were separated using the least significance difference (LSD) Duncan's multiple range tests.

RESULTS AND DISCUSSION

Rumen fermentation, ruminal microbial population, and microbial nitrogen syntheses

Rumen fluid fermentation parameters are shown in Table 2. The results obtained from rumen pH were not influenced by the experimental feed additives. The control group and *Yucca* supplemented groups revealed significant declines in rumen pH compared to other groups. The results of short-chain fatty acid (SCFA) and ammonia N concentration are presented in Table 2. The results indicated that the total concentration of short-chain fatty acid (SCFA), acetate

concentrations, and a percentage of acetic: propionic (A: P) were higher (P< 0.05) for cows supplemented with probiotics (*S. cerevisiae*). In comparison to the other groups, *Yucca* supplementation had a significantly lower (P < 0.05) effect on total SCFA and decreased acetate propionate. The rumen fluid ammonia N concentrations revealed a significant (P < 0.05) decrease in cows supplemented with probiotics (*S. cerevisiae)*. However, the lowest (P < 0.05) value was recorded for the cows supplemented with *Yucca*. The reduction was approximately about 19.47% compared to the results obtained from the control group. The green seaweed additives (*Ulva lactuca*) did not affect rumen ammonia N concentration or total SCFA. The effects of additives on the ruminal population of total protozoans and total bacteria are shown in Table 2. Supplementation of *Yucca* harmed the population of protozoans (P < 0.05) while the populations of total bacteria tend to increase without significant differences. Furthermore, additive seaweeds revealed a significant decrease in the rumen bacterial and protozoa populations. The addition of live yeast (*S. cerevisiae*) did not have any significant effect on the total protozoa or total bacteria population. The results obtained from the microbial nitrogen are shown in Table 2. The results illustrate that the experimental feed additives had a positive effect on microbial nitrogen synthesis and ranged from 70.05 to 74.48 g/ day.

In-Vitro TOTAL GAS AND METHANE PRODUCTION AND *IN VITRO* DM AND OM DIGESTIBILITY

Figure 1 displays the effects of feed additives on the accumulated gas production corrected for blank. The cumulative volume of gas production increased with an increase in incubation time. The values of cumulative gas after 96 hours ranged from 61.1 ml to 48.3 ml per 200 mg of DM for the control and the green seaweed, respectively. While the results of total gas production are presented in Table 3. The results indicated that the experimental feed additives had a significant (P < 0.05) decrease in total gas production and the lowest (P < 0.05) value was recorded with supplemented green seaweed. Also, the rates of gas production were influenced by feed additives and reduction (P < 0.05) the value. Methane production was strongly affected by green seaweed (P < 0.05) after 24–48 hours. The results of methane emission after 24-48 hours incubation are presented in Table 3 as well Figure 2 displays the effects of experimental additives on the methane production at 12, 24, 48, and 72 hours. The experimental feed additives had a decrease in methane production after 24 hours of approximately 12.2, 19, and 22.9% for probiotic (*S. cerevisiae*), *Yucca,* or green seaweed, respectively. Furthermore, after 48 hours the reduction was 12.6%, 21.6%, and 25.16% for (S. *cerevisiae*), yucca, and green seaweed, respectively.

Table 3 showed the effects of probiotics (*S. cerevisiae*), green seaweed, and *Yucca* feed additives on *in-vitro* DM digestibility (IVDMD) and *in-vitro* DM digestibility (IVOMD). The values of IVDMD and IVOMD increased (P< 0.05) by 6.2% and 6.5% with probiotics (*S. cerevisiae)* respectively. Moreover, green seaweed recorded an increase (P < 0.05) of IVDMD and IVOMD by 3.4% and 4.5%, respectively. While *Yucca* resulted in a significant reduction (P < 0.05) of IVDMD and IVOMD of 2.4% and 2.5%, respectively.

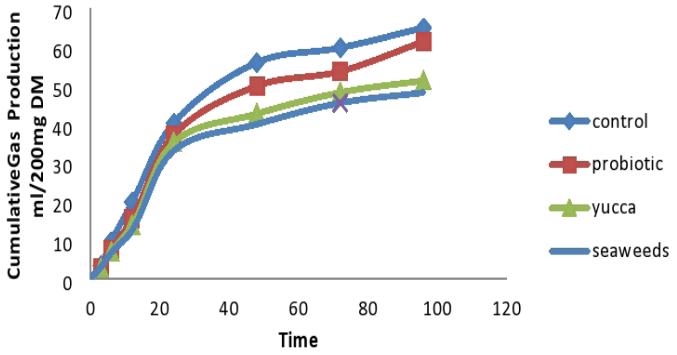

Figure 1: Gas production ml/200mg DM.

Table 2: Rumen fermentation, total protozoa, total bacteria and microbial nitrogen of lactating crossbred Friesian cows feed rations.

Item	Control	Probiotics	Yucca	Seaweeds	SEM	P-value
In vivo Ruminal pH	6.53	6.60	6.54	6.57	0.38	0.095
In vivo Ruminal NH_3-N (mg L^{-1})	14.17^{a}	12.98^{b}	11.41^{c}	13.86^{ab}	0.54	0.016
In vivo Total SCFA (mmol L^{-1})	103.10^{b}	105.51^{a}	99.71^{c}	102.80^{b}	7.54	0.038
Acetic, C_2 (ml/100ml)	62.13^{b}	64.91^{a}	58.67^{c}	62.36^{b}	3.57	0.005
Propionic,C_3 (ml/100ml)	24.88^{b}	25.43^{ab}	26.45^{a}	25.11^{ab}	0.69	0.017
Butyric, C_4 (ml/100ml^{l})	12.33^{b}	13.84^{a}	11.60^{c}	13.58^{a}	0.44	0.043
C2:C3 ratio	2.49^{a}	2.55^{a}	2.21^{b}	2.48^{a}	0.06	0.008
Total protozoa 10^4/ml rumen liquid	6.67^{a}	6.68^{a}	6.01^{b}	6.25^{ab}	0.72	0.021
Total bacteria 10^9/ml rumen liquid	8.25	8.26	8.29	8.01	0.86	0.065
*PD(mmol/day)	124.73^{b}	129.12^{a}	129.90^{a}	128.1^{a}	4.87	0.046
microbial nitrogen g/day	70.05^{b}	73.81^{a}	74.48^{a}	73.10^{a}	0.57	0.023

SEM, standard error of the mean. * PD: purine derivatives (allantoin and uric acid in urine). a, b, c: means in the same row with different superscripts are differ significantly (P< 0.05).

Table 3: Total gas production, methane production and *in vitro* DM and OM digestibility.

Item	Control	Probiotics	Yucca	Seaweeds	SEM	P-value
Rates of gas production	0.0704^{a}	0.0673^{ab}	0.0603^{b}	0.0591^{b}	0.025	0.0051
Total gas production	56.07^{a}	52.82^{b}	48.21^{c}	47.69^{c}	0.57	0.017
Methane production at 24 h	7.80^{a}	6.85^{b}	6.32^{bc}	6.01^{c}	0.19	0.011
Methane production at 48h	9.10^{a}	7.95^{b}	7.13^{c}	6.82^{d}	0.17	0.028
IVDMD*	50.22^{b}	53.32^{a}	49.02^{b}	51.92^{ab}	0.24	0.005
IVOMD**	52.04^{b}	55.41^{a}	50.75^{b}	54.36^{a}	0. 16	0.007

SEM, standard error of the mean. a, b, c: means in the same row with different superscripts are differ significantly (P< 0.05). **in vitro* dry matter digestibility. ***in vitro* organic matter digestibility.

Table 4: Dry matter intake, Milk yield and milk composition of lactating crossbred Friesian cows feed rations.(mean ± SE).

Item	Control	Probiotics	Yucca	Seaweed	SEM	P-value
*DMI, kg/d	17.50^{a}	18.02^{a}	16.90^{b}	17.87^{a}	0.72	0.024
Milk yield kg/d	18.12^{b}	19.47^{a}	17.67^{b}	19.10^{a}	0.81	0.038
4 % FCM	16.95^{b}	18.39^{a}	16.37^{b}	17.95^{a}	0.93	0.017
Fat, kg/d	0.65 ^{b}	0.71^{a}	0.62^{b}	0.69^{a}	0.67	0.005
Milk composition (%)						
Total solids	11.68	11.77	11.70	11.80	0.45	0.720
Fat	3.57^{ab}	3.63^{a}	3.51^{b}	3.60^{a}	0.08	0.008
Protein	3.16^{b}	3.18^{b}	3.23^{a}	3.19^{b}	0.26	0.003
Lactose	4.23	4.26	4.25	4.27	0.18	0.077
Ash	0.72	0.70	0.71	0.74	0.04	0.064
**SCC × 10^{3}/mL	93.2^{a}	82.1^{b}	79.6^{c}	77.8^{c}	5.31	0.041
Saponin ng/ml	--	--	0.86	--	--	--
Tannins ng/ml	--	--	--	0.48	--	--
Milk energy content (kcal/kg)	680.72^{b}	688.58^{a}	680.08^{b}	686.80^{a}	11.52	0.038
Energy-corrected milk (ECM), kg/d	16.82^{b}	18.25^{a}	16.37^{b}	17.85^{ab}	0.67	0.012

SEM, standard error of the mean. a, b, c: means in the same row with different superscripts are differ significantly (P< 0.05). * Dry matter intake. ** Somatic cell count.

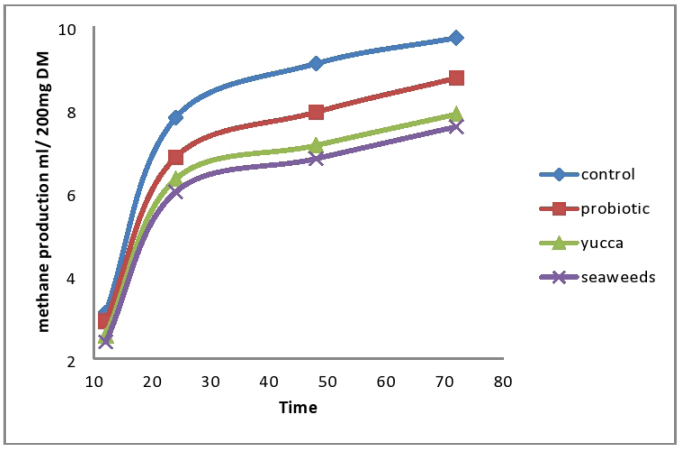

Figure 2: Methane production ml/200mg DM.

Dry matter intake, mike yield, and milk composition

The effect of feed additives on dry matter intake (DMI) is presented in Table 4. Dry matter intake (DMI) increased 2.97% and 2.11% for probiotics (*S. cerevisiae*) and green seaweed supplementations, respectively without significant differences. While the lowest (P < 0.05) feed intake was recorded with cows supplemented with *Yucca* by 3.43%. Results of the daily milk production, FCM (4%) production, and milk composition are presented in Table 4. The dairy cows fed diets supplemented with *S. cerevisiae* or green seaweed showed an increased milk yield and MCF4% (P < 0.05) than other groups. Despite, the dairy cows supplemented with *Yucca* recorded the lowest (P < 0.05) actual milk production and 4% FCM yield, the milk protein composition was significantly (P < 0.05) increased compared to other groups. The additives were reflected in the milk fat composition Table 4. Probiotic (S. *cerevisiae*) significantly (P < 0.05) increased milk fat, whereas the additive green seaweed had a negligible effect. *Yucca* had the lowest (P < 0.05) milk fat % value. No significant differences (P < 0.05) were found for milk lactose (%) among rations. The experimental feed additives showed a

significant (P < 0.05) difference in SCC reduction.

The values for hematological tests and the values for serum biochemical tests (liver and kidney function) in Table 5 showed that the experimental feed additives did not affect hematological values and enzymatic activity of the kidney and liver. No significant differences were found among groups of fed experimental diets for any of the hematological and biochemical tests except urea was significantly (P < 0.05) decreased by the *Yucca*.

Rumen fermentation, ruminal microbial population, and microbial nitrogen syntheses

Ruminal pH is an important indicator of normal rumen function. Feed additives did not affect the rumen pH. The change was ranging from (6.53 to 6.60). This may be attributed to the rumen having the buffering capacity to keep pH in the normal range for active cellulolytic bacteria, without any unfavorable fermentation in the rumen. These results were in agreement with results obtained by Ambriz et al. (2017), Canul-Solis et al. (2017), and Abderzak Lettat et al. (2012) who found that adding probiotics to ruminant rations were more effective in stabilizing rumen pH via stimulation lactate-utilizing bacteria. Also, Wina et al. (2005) found that administering *Yucca* to ruminants hasn't impacted rumen pH. On the other hand, additive *Yucca* leads to a decrease in the ammonia nitrogen concentration. These results were in agreement with Singer et al. (2008) concluded that *Yucca* extracts contain sar-saponin, (steroidal saponins) which can impact rumen fermentation as a result of a reduction of protozoal numbers (defaunation) that contributes from 10 to 40% of the total rumen nitrogen Van Soest et al. (1991), and led to decreased rumen ammonia concentrations and increased microbial nitrogen. Furthermore, studies by Morales et al. (2107) and Guyader et al. (2017) have demonstrated that *Yucca* extracts decrease total SCFA production, acetate proportions, and increase propionate as a result of the defaunation effect. Patra (2010) observed that saponin can inhibit the protozoa population and thus reduce the digestibility of DM, organic matter, and fiber whereas the protozoa have an important role in the digestion of fiber fraction, which is reflected on acetate. Some studies reported that saponin supplementation led to decreased ruminal fermentation parameters Guyader et al. (2017) and Singer et al. (2008). Whereas other studies found no effect of saponin on ruminal fermentation like Hu et al. (2006) and Guo et al. (2008). On the other hand, probiotic (*S. cerevisiae*) supplementation had been enhancement the rumen function and led to an increase of individual and total (SCFA) concentration and acetate: Propionate, the results were in consent with Jiang et al. (2017) and Kampanat et al. (2021), who illustrated increases the concentrations of total (SCFA) and acetate at the expense of propionate with live yeast supplementation. Pinloche et al. (2013) found that live *S. cerevisiae* was able to stimulate cellulolytic rumen bacteria and promote most of the rumen fermentation and increase the acetate. Furthermore, the addition of probiotics (*S. cerevisiae*) led to a reduction of ruminal NH_3-N concentrations, this result was in agreement with Soliman et al. (2016) and Firkins and Morrison (2007), and who suggested that lower NH_3-N concentrations have been shown due to implicating to growth and increased rumen bacteria that consume NH_3-N in the rumen pool. This hypothesis corresponds with Hristov et al. (2010) who found that when live yeast (*S. cerevisiae*) is supplemented with ruminant, it improves the utilization of ruminal ammonia-N, and increases cellulolytic bacteria that have a high preference for ammonia as their N source. The results obtained from the addition of green seaweed (*Ulva lactuca*) can be discussed. They contain Alginic acid, a polysaccharide compound that has been demonstrated to be readily degraded by the rumen microbes, and produce SCFA that is used by microbes for growth Castillo-González et al. (2014), might be speculated that a negligible reduction of ammonia-N and SCFA may be due to synchronization between SCFA as a source of energy and NH_3-N used by rumen microbes. These results concur with Moneda et al. (2019) while Margarida et al. (2016) reported that supplementing seaweed to ruminant diets did not affect rumen ammonia concentration or total SCFA. Feed additives stimulate microbial protein synthesis. Furthermore, there are correlations between increasing organic matter digestibility (OMD) and increasing microbial protein so OMD provides more nutrients for the growth of the microbes Liu et al. (2019). The effect of supplemented *Yucca* on increasing microbial protein is due to the effect of saponin, which causes defaunation.

Table 5: Haematology, enzymatic liver and kidney function of lactating crossbred Friesian cows feed rations. (mean±SE).

Item	Control	Probiotic	Yucca	Seaweeds	SEM	P-value
White blood cell (10^3/µL)	7.84	7.72	7.62	7.90	0.38	0.201
Red blood cell (10^6/µL)	8.33	8.42	8.463	8.75	0.42	0.744
Hemoglobin (g/dL)	12.41	12.55	12.34	12.64	0.26	0.160
*AST U/L	79.45	77.82	78.11	77.61	0.09	0.136
**ALT U/L	11.57	11.34	11.63	10.19	0.11	0.094
creatinine (g/dL)	0.78	0.88	0.93	0.86	1.06	0.088
Urea (mg/dL)	19.02[a]	19.25[a]	15.74[b]	17.25[ab]	1.05	0.015

* Aspartate aminotransferase. ** Alanine aminotransferase.

In-vitro total gas and methane production and *in-vitro* DM and OM digestibility

The effects of feed additives probiotic (*S. cerevisiae*), *Yucca*, and green seaweed on rumen total gas production and CH_4 production were examined in *in-vitro* conditions. The results revealed that *S. cerevisiae* contributed to a decrease in gas and methane production. Since *S. cerevisiae* might stimulate ruminal acetogenic bacteria, which produces acetate from CO_2 and H_2 (Emilio 2020). Chaucheyras et al. (2008) and Weinberg (2003) reported that *S. cerevisiae* produces many important fermentation metabolites and contains an important mineral and enzymes that represent essential growth factors for lactic acid fermenting bacterial species such as *Megasphaera elsdenii* and enhanced hydrogen utilization of acetic acid-producing bacteria. So, it can be predicted that the increment of metabolic hydrogen led to a reduction of ruminal methanogenesis with probiotics (*S. cerevisiae*). Chaucheyras et al. (2008) reported that the yeast live cells can persevere for as long as 24–30 hours in the rumen and demonstrated that the yeast viability in the rumen plays a role in the effects observed on the rumen microflora. Similarly, additives such as *Yucca* statistically reduce total gas production and methane attributed to saponins containing a complex compound of sarsaponins that react with steroid in the protozoal cell membrane, causing membrane breakdown, cell lysis, and death (saponins have surfactant properties attached to sterols in the protozoa cell membrane). Reductions in ruminal protozoa counts were in agreement with Eugène et al. (2004), Guyader et al. (2014), and Zongjun et al. (2018) who suggested that defaunation generally led to a decrease in rumen methanogenesis. Hess et al. (2003) and Santoso et al. (2004) found that about 25% of ruminal methanogenesis is associated with protozoa. Therefore, adding *Yucca* leads to inhibition of methanogens that interact with other ruminal microbes, including bacteria and fungi, through interspecies H_2 transfer hence coming to stop hydrogen transferred by Patra et al. (2017). These results were consistent with Hess et al (2003) and Santoso et al. (2004) who reported that saponins can act directly on methanogens and protozoa to reduce total gas production and methane production.

A significant decrease in gas production and CH_4 production were observed in this study when using green seaweed. Because, green seaweed has a high proportion of phenol compounds, and essential oils volatile (Kumar and Navaratnam, 2013; Byeng et al., 2021; Dubois et al., 2013) from a large diversity of secondary metabolites. Tannins, flavonoid, and essential oils are the main secondary metabolites of green seaweed; may inhibit methane production by their main effect on a specific rumen bacterial community and there could be variations in their toxicity towards certain rumen bacteria and ciliate protozoa Pellikaan et al. (2011), Patra and Saxena (2011) and hence decreased methane emissions. The secondary plant metabolites of seaweeds act directly on methanogenic bacteria cells due to the structure and properties contained therein and their anti-methanogenic effect in the rumen that may be related to the presence of organic halogens (Cieslak et al., 2013) are incorporated into various components such as terpenoids and phenylpropanoids, demonstrated that have inhibition of methanogenesis. Byeng et al. (2021) and Tsiplakou et al. (2017) reported that applied seaweeds as feed additives in ruminant diets reduce enteric methane emission during rumen fermentation processes. Generally, all the additives in this experiment may cause different changes in the microbial community and thus the fermentation processes in the rumen and then reduce the gas production and methane production.

The effect of experimental feed additives in terms of IVDMD and IVOMD have observed relatively high values for green seaweed, which contain micronutrients (essential nutrients, especially trace elements) and can improve the digestibility of ruminants, these results were in agreement with Molina et al. (2017) and Anele et al (2016). Who reported that adding green seaweed to ruminant diets led to an increase ($P < 0.05$) in the IVDMD. As well, supplementation with probiotics (*S. cerevisiae*) had improved the *in vitro* DM and OM digestibility. Boyd et al. (2011) and Malik and Singh (2009) recorded higher values of IVDMD and IVOMD by adding active yeast to ruminate, which may be due to stimulating rumen bacteria growth and fermentation Stein et al. (2006), consequently enhanced DM and OM digestibility. In contrast, the results of additive *Yucca* led to a significant decrease in IVDMD and IVOMD. Several studies observing the decline in IVDMD and IVOMD due to the addition of *Yucca* to ruminate diets have been reported by Jadhav et al. (2016) and Yogianto et al. (2014). Furthermore, Agarwal et al. (2006) and Hess et al. (2003) speculated that saponins might reduce the activity of digestibility organic matter and neutral detergent fiber (NDF) as a result of lowering rumen protozoa, thus leading to lower not only the digestibility of fiber faction but other nutrients as well.

Dry matter intake, mike yield, and milk composition

Dietary supplementation with probiotics (*S. cerevisiae*) or green seaweeds for dairy cows had no effects on dry matter feed intake (DMI) this results following (Kumar and Navaratnam, 2013) and Alshanbari et al. (2020) while adding *Yucca* to dairy cows diet had a negative effect ($P > 0.05$) of DMI that were attributed to the presence of steroidal saponins compounds that reduced palatability and nutrient digestibility (Hristov et al. 2010; Lovett et al., 2006).

Dairy cows fed diets supplemented with probiotics (*S. cerevisiae*) had an increased milk yield, MCF 4% and fat % of 7.5, 8.5, and 1.71%, respectively, these results are consent with Omar et al. (2020), Anjum et al. (2018), Alshanbari et al. (2020) who reported that the incorporation of probiotic yeast in dairy cows trend towards improved milk production ranging from 6 to 12%. A positive effect of *S. cerevisiae* additive on fat % and MCF4% are linked to the stimulation of cellulolytic bacteria, and a preferred orientation of fermentation to acetic acid production. The addition of green seaweeds increased milk yield, MCF4%, and fat % up to 5.4, 6.0, and 0.84%, respectively. This result was in agreement with (Ead and Eman, 2011; Hostens et al., 2011; Cvetkovic et al., 2004). The positive impact on milk production has been reported by the benefits of additives like green seaweed effects on dairy cows' diets that provide essential micronutrients (Eric et al., 2021; Bendary et al., 2013) also, contains many biologically active compounds such as betaine (trimethylglycine). Fernández et al. (2009a) demonstrated that supplementing betaine to lactating dairy cows can increase milk yield and milk fat content, also contains pigment, provitamins, vitamins, and growth factors, as well as all the basic nutrients (Holdt, 2011) that promote animals immunity and might be responsible for increased milk production. However, it was reported that the negative effects of supplemented *yucca* on milk yield, 4% FCM, and fat % could be attributed to decreased nutrient digestibility and depression of DMI. These results are consistent with Anantasook et al. (2014), Holtshausen et al. (2009), and Wilson et al. (1998) which found an increase in propionate concentrations and/or reductions in DMI. Also, Patra and Saxena (2011) observed that lowering the acetate: Propionate ratio would result in a reduction of 4% FCM and % fat. but, in contrast to the results obtained by Cohen-Zinder et al. (2016) and Moyosore et al. (2019), found a significant increase in milk yield after 12 weeks, as a consequence of reduced rumen ammonia, which led to decreased excretion of ammonia in the form of urea through urine or as nitrogen in feces, thus reducing nitrogen odor in manure and perhaps improving milk yield.

Despite the significant decline in dairy cows fed diets supplemented with yucca in terms of milk yield and milk fat, but the protein milk yield has increased compared with those feed diets supplemented with seaweeds or *S. cerevisiae* compared to other groups.

Milk protein has increased in cows fed *yucca*-supplemented diets compared to those fed seaweed or *S. cerevisiae*-supplemented diets. while decreasing milk yield and milk fat.

The dairy industry used SCC as a monitor hygienic milk quality, increase SCC lead to a change in milk composition, and causes economic losses in the dairy industry Wickström et al. (2009), our studies, all animals recorded normal values of SCC, but the feed additives led to a greater reduction of SCC in milk by 16.7, 14.4 and 11.9% for *Yucca*, seaweed, and *S. cerevisiae*, respectively. That may be due to biological components in experimental additives enhancing immune function and overall animal health.

There have been no reports of side effects or abnormal complaints in the cow's supplemented feed additives containing saponin or tannins, *Yucca*, and seaweed, respectively, or in its calves.

Hematology, enzymatic liver, and kidney function

In the present study, the values for hematologic and biochemical tests (liver and kidney function) as shown in Table 5 revealed that the experimental feed additives did not affect hematologic test values and enzymatic activities values of kidney function and liver. Insignificant differences among groups fed experimental diets for any of the hematologic or serum biochemical tests except that blood urea was significantly ($P < 0.05$) decreased by the *Yucca* additive but the value was in the normal range. Narirat et al. (2021) have been speculated that urea may reflect the ratio of dietary crude protein to rumen-fermentable nitrogen compounds and post ruminal protein supply, a lower blood urea (BU) value was found in cows supplemented with yucca. Lower BU is usually associated with a lower ruminal NH_3-N This finding confirms the ability of yucca to reduce BU levels, which supported its ability to reduce the ruminal NH_3-N concentration.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates the potential effect of feed additives that can be beneficial for increasing ruminant performance and contribute to overcoming the methane emissions from ruminants. Thus, a new composition of a mixture of these feed additives may have arrived at that achieves the maximum benefit. Future work will be later necessary to investigate further the role of probiotics (live yeasts), *Yucca*, and seaweeds as an ecological tool to control methane emissions in the rumen without effect on animal performances.

NOVELTY STATEMENT

Utilizing the green seaweeds that are abundant on the Egyptian coasts as a source of feed additives that contribute to reducing methane emissions and enhancing animal production, after comparing them with the most commonly used feed additives for dairy cows in order to

reduce methane or increase production.

Conflict of interest

The author has declared no conflict of interest.

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