International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2020.88.98

Research Article
Effect of Biological Extract Supplementation on Milk Yield and Rumen Fermentation in Dairy Cows

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Abstract

Background and Objective: Recently use of extracts plants in animal nutrition as feed additives and natural alternatives for antibiotic, for improve feed efficiency and enhancing livestock productivity has increased. The current study was designed to determine the effect of oral administration of fruit and vegetable juice (FVJ) as natural additives on the productivity of dairy cows. **Materials and Methods:** In this study eighteen lactating crossbred Holstein Friesian cows were used in three similar groups (6 cows/group): 100 mL water/cow (control group). Group 2 and 3 were supplemented with 50 mL FVJ/cow (50 FVJ) (Group 2) and 100 mL FVJ/cow (100 FVJ) (Group 3) for 70 days. During experiment period parameters of milk, digestibility, fermentation, gas production, methane production and blood was evaluated. **Results:** The study revealed increased in digestibility coefficients, milk yield, 4% FCM and milk composition in Group 2 and 3. Concurrently ruminal fluid fermentation of total VFAs, acetic acid and ammonia-N concentrations had decreased. Also, cow's supplementation with FVJ had a favorable effect to reduce cumulative gas production and methane compared to the control group. Moreover, the treatment with (100 FVJ) showed the lowest ($p<0.05$) *in vitro* dry matter disappearance (IVDMD) and total gas production compared with treated (50 FVJ) or control group. The treatments (50 FVJ) and (100 FVJ) had enhanced the hematological parameters (RBC, Hb, WBCs and PCV) in comparison to the control group. Simultaneously, cows supplemented with FVJ reduced liver enzymes compared with those in control group. The evaluation of (ALT and AST) showed a significant decrease for cow treatments by FVJ compared to the control. **Conclusion:** It concluded that supplementation of FVJ (50 or 100 mL/cow/day) to dairy cow improved the feed digestibility, rumen fermentation and productive performance.

Key words: Lemon onion and garlic juice, milk yield, gas production, digestibility, extract biological, blood constituents and dairy cows

Citation: S.M. Soliman, A.A. Hassan, Neamat I. Bassuony and A.M. El-Morsy, 2020. Effect of biological extract supplementation on milk yield and rumen fermentation in dairy cows. Int. J. Dairy Sci., 15: 88-98.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many plants produce secondary metabolites that may be useful as feed additives because of their biologically active constituents[1]. Plant-derived bioactive compounds such as phenolics, flavonoids compounds, essential oils and allicin are naturally occurring secondary metabolites and volatile components considered to be valid sources of phytonutrients and immunity enhancers, increasing antioxidant activity and have antimicrobial properties that are effective against a wide variety of microorganisms[2]. Additionally, the secondary metabolites can be used to modify rumen fermentation. The purpose of rumen modifications is to maximize the efficiency of converting feed animal products by manipulating the ecosystems of ruminal microbes, without leaving behind a negative impact on the environment[3]. Recently used these plants rich with secondary metabolites or herbal extracts as animal feed additives after was proven a safe and beneficial[4,5]. Methane emissions from ruminant animals are currently estimated to be 100 million tons each year making them significant contributors to global warming, imposing an environmental burden that cannot be ignored. The excretion of methane from ruminants is a problem not only in respect of greenhouse gas emissions, but it also represents a loss of 2-15% of gross energy intake[6]. Certain plant extracts such as garlic and onion, containing plant secondary compounds can reduce either CH_4 emissions or NH_3 formation (or both) without compromising the general fermentation of nutrients in the rumen[7]. Also, they seem to reduce measures of acetate/propionate.

The citrus fruit is a valuable component of the fruit food group and one of the most widely distributed crops in the world. Dried citrus pulp is a valuable edible and raw material containing various soluble and insoluble carbohydrate polymers that are used as animal feed[8]. Furthermore, they contain active compounds such as ascorbic acid, flavonoids and phenolic compounds[9]. Citrus pulp contains limonene; which has been reported to have anti-methanogenic properties *in vitro*[10]. The total phenolic contents for citrus pulp, garlic and onion expressed as mg of gallic acid equivalents (GAE) g^{-1} were 8.25, 25.3 and 28.1, respectively[11]. Garlic (*Allium sativum*) and garlic oil have a wide spectrum of effects ranging from antimicrobial, antioxidant and anticarcinogenic properties to beneficial effects on the cardiovascular and immune systems[12]. The onion, a versatile vegetable of the *Allium* genus, is appreciated worldwide and is the second most studied plant belonging to this genus after garlic; well known for its useful constituents such as sulphur compounds, phenols and flavonoids that have major roles in being antimicrobial and antioxidant as well as improving metabolites activities[7].

Many studies confirmed the positive effects of flavonoid-rich plant extracts to reduction methane emission methane emission and changes in microbial populations such as protozoa and methanogen, thus improving ruminal fermentation of dairy cows with increasing milk yield and protecting from ruminal acidosis[13]. Thus, the rumen fluid and any associated compound may have a short survival time in the rumen and a high probability of reaching the intestines of the host animal. Once the plant nutrients reach the intestinal sites, they can be rapidly absorbed and have positive physiological characteristics[14]. Improvements in digestibility and immune response can all together improve animal health, milk production and feed efficiency in dairy cattle. In recent years, there is increasing research on natural plants that produce secondary metabolites, the relationship between animal products through rumen modification and effects on animal production without any side effects on the health of the individual animal. Therefore, the aim of this study was to evaluate the effect of patent fruit and vegetable juices (including lemons, garlic and onion) on milk production, milk composition, some blood parameters, rumen fermentation and methane production in dairy cows.

MATERIALS AND METHODS

Animals, housing and feeding: This study was carried out at Noubaria Station, Animal Production Research Institute in December, 2016. This experiment designed to determine the effects of the oral supplementation of p, juices (FVJ) on productivity of dairy cows. This juices were supplied by the Regional Centre for Food and Feed, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt (Patent No.: 27392, Cairo, Egypt 2016). Prior to the study, the FVJ was tested and several components are found like (2,5-Di-tert-butylhydroquinone (DBHQ), Allicin, Allyl mercaptan, Flavonoids, Quercetin, hydroxybenzoic acid, D-Limonene, Aspartic acid, S-2-aminoethyl cysteine, DL-3-aminobutyric acid, Cis-4,7,10,13, 16,19-Docosahexaenoic acid, also contained amino acid methionine, lysine, phenylalanine, serine and as well as minerals (copper, calcium, potassium, magnesium, zinc, iron, sulphur, selenium and chromium).

Table 1: Ingredients and chemical composition of the total mixed diets

Items	TMR
Ingredients (g kg^{-1} DM)	
Corn silage	400.0
Wheat straw	45.0
Alfalfa hay	155.0
Wheat grain ground	131.0
Wheat bran	95.0
Soybean meal ground (44% CP)	154.0
Calcium carbonate	13.0
Salt (NaCl)	2.0
Vitamin-mineral premix*	5.0
Total (%)	1000 .0
Chemical composition (g kg^{-1} DM)	
Dry matter	643.8
Crude protein	140.3
Ether extract	29.3
Neutral detergent fiber	395.1
Acid detergent fiber	234.7
Acid detergent lignin	36.1
NFC	373.6
Starch	254.8
Ash	61.7

*Supplied per kilogram of premix (Kavimix VM, Kartal Kimya A.S., Gebze, Turkey): Vitamin A: 12 000 000 IU, Vitamin D3: 3000000 IU, Vitamin E: 30 g, Mn: 50 g, Fe: 50 g, Zn: 50 g, Cu: 10 g, I: 0.8 g, Co: 0.1 g, Se: 0.15 g, Antioxidant: 10 g, NFC: Nonfiber carbohydrate (%), calculated as: 100-[(NDF (%)+CP (%)+EE (%)+ash (%)], TMR: Total mix ration

Eighteen multiparous lactating crossbred Friesian cows in early lactation (30±3 days in milk) were assigned randomly to three treatments (6 cows/each treatment) stratified by live body weight (544±5.1 kg). The cows were housed individually in soil-surfaced tie stalls, under shade, without any bedding and with access to water. Cows were fed a TMR to meet their nutrient requirements according to NRC[15] recommendations. The nutrient contents of feed ingredients are shown in Table 1. Individual cows were weighed on a digital multi-purpose platform scale (PS-2000 Platform Scale, Salter Brecknell, Fairmont, MN, USA) at the 1st, 35th and 70th days of the experiment. The cow's supplementation oral dose was by either plain water (sham treatment, 100 mL water/cow as a control group) or the equivalent of 50 and 100 mL/cow of the fruit and vegetable juices (50 FVJ) and (100 FVJ) respectively, for 70 days. A preliminary period was set to determine the best proportions of doses that do not affect the smell of milk. Diets were offered twice a day at 07:00 and 19:00 pm. Samples of TMR were taken daily, composited weekly, dried at 60°C in a forced-air oven for 48 h (AOAC)[16] and stored for chemical analysis.

Milk sampling and milk composition: Cows were machine milked twice daily at 06:00 and 18:00 pm and samples (100 mL L^{-1} 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 were analyzed for total solids, fat, protein and ash according to methods of Ling[17], 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^{-1}) of milk. Fat corrected milk (4%) was calculated according to Gaines and Davidson[18] using the following equation:

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

where, M is the milk yield and F is the fat percentage.

Milk energy value (E) was calculated according to Kleiber[19] by using the following equation:

$$E\ (kcal\ kg^{-1}) = (Fat\ (\%)\times 92)+(Protein\ (\%)\times 58.6)+(Lactose\ (\%)\times 39.5)$$

The milk energy output (kcal/day) was then calculated as:

$$Milk\ energy\ (kcal\ kg^{-1})\times Milk\ yield\ (kg/day)$$

Energy-corrected milk (ECM) was calculated according to Sjaunja *et al.*[20] as following equation:

$$ECM\ (kg/day) = \frac{Milk\ production^{*}\ (^{*}383\%\ fat+^{*}242\%\ protein+783.2)}{3140}$$

Milk samples for somatic cell count (SCC) determination was collected at the 8th week postpartum and the analysis was done according to the method of Hirst *et al.*[21].

Feed intake and nutrient digestibility: Feed intake was recorded daily by weighing the offered rations and refusals from the previous day. At the last week of the first and second periods of the experiment (30-35 and 65-70 days), a nutrient digestibility trial was carried out in which acid insoluble ash was used as an internal indigestibility marker and coefficients of digestion were calculated according to Ferret *et al.*[22]. Faecal grab samples were collected from each group twice daily and then dried at 60°C in a forced-air oven for 48 h. Dried rations and faecal samples were ground to pass a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and analyzed for dry matter (DM), ash, crude protein (CP) and ether extracts (EE) according to AOAC[16] official methods. Neutral detergent fibre (NDF), acid detergent fiber (ADF) and lignin were determined by the procedure of Van Soest *et al.*[23]. Concentrations (g kg^{-1} DM) of non-fiber carbohydrates = 1000-(NDF+CP+EE+ash), cellulose (cellulose = ADF-lignin) and hemicelluloses (hemicellulose = NDF-ADF).

Sampling and analysis of rumen fluid: Ruminal fluid contents were sampled at 0 times before feeding and at 3 and 6 h after the morning feeding using stomach tubing from cows from 35-37 days. Approximately 100 mL of rumen fluid were collected from each treatment (the same cows used in the digestibility ruminal fluid was mixed with 0.2 mL of a solution containing 250 g of metaphosphoric acid/L for TVFA's analysis by titration, after steam distillation of a 4 mL sample, by the method of Annison[24]. Samples were stored at -20°C until analyses. Concentration and molar proportions of individual VFA were measured by gas-liquid chromatography (model 5890, HP, Little Falls, DE, USA). The separation process was carried out with a capillary column (30 m×0.25 mm internal diameter, 1 m film thickness, Supelco Nukol; Sigma-Aldrich, ON, Canada) and with flame ionization detection. The column temperature was adjusted to 100°C for 1 min and then increased by 20°C min^{-1} to 140°C and then by 8°C min^{-1} to 200°C and held at this temperature for 5 min. Helium was used as the carrier gas.

Measurement of gas production: 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, 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 as described by Menke and Steingass[25]. 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 and 48 h of incubation. 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.*[26]:

$$EXP0\ V = VF\ (1\text{-}exp\ (-kt))$$

Where, V is the cumulative gas production (mL) at different incubation times, VF is the final asymptotic gas volume, k is the fractional rate of gas production, t is the incubation time (h). The fractional rate (μ, h^{-1}) calculate according to equation (ln (0.5)/K) K: the time at VF/2. After 48 h incubation, methane was measured by taken samples 1 mL from headspace gas from each syringes by evacuated vials and injecting into gas chromatography (GC) with flame-ionization detection expressed as mL of CH_4 g^{-1} of DM disappeared. After gas was sampled for CH_4 and total gas production was measured, the fermentation syringes were opened and the pH of the culture was measured using a pH meter. At the end of the fermentation period, the fermented residues were filtered into pre-weighed filter crucibles (porosity P160; British Standard grade 1), dried for 24 h at 105°C and weighed and *in vitro* dry matter disappearance (IVDMD) was calculated according to Tilley and Terry[27] by using the following equation:

$$IVDMD\ (\%) = \frac{\text{Sample weight-(Residue weight (sample after incubation)-Blank)}}{\text{Sample weight}} \times 100$$

Sampling and analysis of blood serum: At the end of the feeding trial, blood samples (10 mL) were taken by venipuncture from the jugular veins using heparinized vacuum tubes and were stored on ice until analysis. Whole blood was subjected to analysis in an automatic hematological analyzer (The scil VET abc, Montpellier, France) within 2 h after sampling. Blood serum is collected into tubes with no additives (red cap BD vacutainer tubes) and allowed to stand at room temperature for 45 min to clot. Samples are then centrifuged the serum remains at the top of the tube immediately after the completion of the centrifuge we transfer the serum directly and prepared for storage 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 colorimeter using commercial kits, according to the Reitman and Frankel method Coles[28]. Kidney function was evaluated by measuring blood urea; creatinine and total protein were measured on a colorimeter using commercial kits, were measured according to the method of Grant *et al.*[29].

Statistical analysis: Data for intake, apparent nutrient digestibility, milk characteristics, ruminal fermentation and blood profiles were analyzed as a completely randomized design with repeated measures using the PROC MIXED procedure of SAS[30] with week as repeated measures and individual animal as the experimental unit. Statistical processes were carried out using the General Linear. The model describing each trait was assumed to be:

$$Y_{ijkl} = \mu + T_i + a\ (T)_{IJ} + WK + E_{ijkl}$$

where, Y_{ijkl} is the parameter under analysis, μ is the overall mean, T_i is the fixed effect of treatment, a $(T)_{IJ}$ is the random effect of animal (j) nested within treatment (i), WK is the fixed effect of week when K = 1, 2,⋯.,8, E_{ijkl} is the random error. Significant differences among means were separated using LSD test according to Duncan[31].

RESULTS

Digestibility coefficients: Data in Table 2 indicated that the cow's oral dosage administration of fruit and vegetable juices at level (50FVJ) displayed significantly ($p<0.05$) increased digestibility of organic matter (OM) and crude protein (CP) and recorded the highest values than those administered (100FVJ) or the control group. While, the digestibility of NDF was showed a significantly linear decrease with increased doses from level 50 FVJ to level 100 FVJ compared to the control group which recorded the highest ($p<0.05$) value. The results obtained from acid detergent fiber (ADF) digestibility did not show any significant differences between the control group and those orally administered by (50-100 FVJ).

Milk yield and milk composition: The results of dry matter intake (DMI) showed no differences significantly among the experimental groups in regard to (DMI) (Table 3). On the other hand, the results of milk yield and composition observed that the cows supplemented at level (50 FVJ) recorded highest significant ($p<0.05$) values of milk yield, 4% FCM and milk composition (total solids, fat and protein) followed by (100 FVJ) while, animals in control groups recorded the lowest values. The results of milk energy content and energy-corrected milk (ECM) showed significantly higher values with animals supplemented by 50 FVJ than those supplemented by 100 FVJ and control groups. Oral supplementation dosages by FVJ at level 50 or 100 displayed decreased somatic cell counts (SCC) in the milk as much as 21.24 and 25.12%, respectively than the control group.

Rumen fermentation, total gas and methane production: Rumen fluid fermentation parameters are shown in Table 4. The results of rumen pH showed no significant difference between among groups, higher rumen pH values were observed with both two levels of FVJ oral dosage supplementation. On the other hand, the effect of oral dosage administration of FVJ at levels (50 or 100) on rumen was that NH_3-N concentration significantly decreased in comparison with the control group, the lowest value was recorded with dose at level 100 VFJ. The oral supplementation at levels 50 or 100 of fruit and vegetable juices recorded lowest values ($p<0.05$) of total FVJ s production and molar acetic proportion, while showing significantly ($p<0.05$) increased molar percentage of propionate and butyrate compared with the control group. The results of *in vitro* studies are shown in Table 4. The oral administration of (FVJ) was observed to be an

Table 2: Digestibility coefficients of lactating crossbred Friesian cows treated with fruit and vegetable juices during lactating period

	Fruit and vegetable juices				
Items	Control	50 FVJ	100 FVJ	SEM	p-value
Digestibility coefficients (%)					
Organic matter	65.34[b]	67.59[a]	64.83[b]	0.56	0.038
Crude protein	63.69[b]	65.77[a]	63.08[b]	0.49	0.022
Neutral detergent fibre	59.78[a]	57.82[b]	56.02[c]	0.66	0.038
Acid detergent fibre	54.89	54.47	53.61	0.39	0.719

[abc]Means in the same row with different superscripts are differ significantly ($p<0.05$)

Table 3: Feed intake, milk yield and its constituent's milk of crossbred Friesian cows fed the oral administration of fruit and vegetable juices during lactating period

	Fruit and vegetable juices				
Items	Control	50 FVJ	100 FVJ	SEM	p-value
TMR intake (kg/head/day)	18.57	18.66	17.98	0.11	0.639
Production (kg/day)					
Milk yield	17.67[b]	18.56[a]	17.79[b]	0.13	0.014
4% FCM	15.97[b]	17.25[a]	16.40[ab]	0.17	0.011
Milk energy content (kcal kg^{-1})	627.98[c]	658.46[a]	641.38[b]	22.06	0.003
The milk energy output (MJ/day)	46.43[b]	51.13[a]	47.74[b]	3.77	0.001
Energy-corrected milk (ECM) (kg/day)	15.99[b]	17.34[a]	16.61[b]	1.04	0.001
Milk composition (%)					
Total solids	10.78[b]	11.16[a]	10.89[ab]	0.19	0.031
Solid not fat	7.42[a]	7.63[b]	7.41[a]	0.05	0.028
Fat	3.36[b]	3.53[a]	3.48[ab]	0.08	0.015
Protein	3.19[c]	3.30[a]	3.21[b]	0.06	0.011
Lactose	3.34	3.35	3.37	0.09	0.026
Somatic cells counts (SCC×10^3 mL)	187.72[a]	147.84[b]	140.56[b]	42.74	0.041

[abc]Means in the same row with different superscripts are differ significantly ($p<0.05$)

Table 4: Rumen fermentation, cumulative gas production and methane production of lactating crossbred Friesian cows through oral administration with fruit and vegetable juices

	Fruit and vegetable juices				
Items	Control	50 FVJ	100 FVJ	SEM	p-value
In vivo ruminal pH	6.54	6.70	6.74	0.09	0.077
In vivo ruminal NH_3-N (mg L^{-1})	14.97[a]	13.63[b]	12.86[c]	0.26	0.031
In vivo total VFAs (mmol L^{-1})	106.30[a]	102.20[b]	98.60[c]	0.14	0.019
Acetic, C_2 (mL/100 mL)	62.73[a]	57.91[b]	55.26[c]	0.29	0.023
Propionic, C_3 (mL/100 mL)	23.88[b]	26.23[a]	25.51[a]	0.13	0.031
Butyric, C_4 (mL/100 mL)	11.33[b]	13.84[a]	13.58[a]	0.74	0.043
C2:C3 ratio	2.63[a]	2.21[b]	2.16[b]	0.29	0.042
IVDMD, cumulative gas production and methane production incubated with rumen fluid *in vitro*					
IVDMD	58.62[a]	54.58[b]	52.29[b]	0.48	0.021
VF (Ml/200 g DM)	54.87[a]	40.44[b]	34.90[c]	0.33	0.041
K (h^{-1})	0.045[b]	0.055[a]	0.057[a]	0.016	0.011
Average CH_4, (mL g^{-1} DM 48 h)	9.09[a]	7.07[b]	6.37[b]	0.17	0.003

[abc]Means in the same row with different superscripts are differ significantly (p<0.05), IVDMD: *In vitro* dry matter digestibility, VF: Final asymptotic gas volume, K: Fractional rate of gas production

Table 5: Complete blood count, measures of enzymatic liver and kidney function of lactating cross Friesian cows treated with fruit and vegetable juices during lactating period

	Fruit and vegetable juices				
Items	Control	50 FVJ	100 FVJ	SEM	p-value
White blood cell (10^3 µL^{-1})	7.84[b]	8.52[a]	8.94[a]	0.38	0.001
Red blood cell (10^6 µL^{-1})	7.44	7.63	7.57	0.42	0.084
Hemoglobin (g dL^{-1})	12.49[b]	13.68[a]	13.96[a]	0.26	0.016
Packed cell volume (%)	31.51[b]	37.80[a]	39.24[a]	0.24	0.011
Platelets (10^3 µL^{-1})	276.00	289.00	282.00	18.64	0.839
Plasma chemical compositions					
AST (U L^{-1})	59.85[a]	48.11[b]	45.78[b]	0.09	0.036
ALT (U L^{-1})	10.57[a]	7.73[b]	7.09[b]	0.11	0.044
Total protein (g dL^{-1})	7.88	8.03	8.36	1.06	0.058
Urea (mg dL^{-1})	11.52[b]	13.74[a]	14.85[a]	0.85	0.014
Creatinine (mg dL^{-1})	0.61[c]	0.77[b]	0.83[a]	0.046	0. 021

[abc]Means in the same row with different superscripts are differ significantly (p<0.05)

improvement on the basic parameters of the *in vitro* incubation of rumen liquor (gas production and methane production). The results showed that the treatment with (FVJ) at either 50 or 100 mL recorded lower values (p<0.05) for cumulative gas and methane production than the control group. Whereas, the results of treatment by (FVJ) recorded lowest values (p<0.05) of *in vitro* dry matter disappearance (IVDMD) compared with the control group.

Blood parameters: Determining blood parameters are helpful in assessing the health status of animals. The results obtained of the effect of oral dose administration on dairy cows with FVJ on blood parameters are shown in Table 5. The results showed that the mean values of the normal values for all cows' oral administration of FVJ or that in the control group. Also, the results have shown that daily supplementation of FVJ either 50 or 100 mL recorded highest (p<0.05) values. Moreover, the results obtained from WBCs indicated that FVJ at different levels had a higher (p<0.05) significant positive effects than the control group. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity in the serum were statistically (p<0.05) much higher for animals in the control group than that with supplementation using FVJ (50 or 100 mL). The results of serum urea and creatinine showed significantly (p<0.05) high for fruit and vegetable juices (50 FVJ), (100 FVJ) over the control groups, while the results obtained of total serum protein was not affected by FVJ supplementation.

DISCUSSION

The current study showed a regression in the digestibility of NDF for cows supplemented by an oral dosage at level (100 FVJ) followed by that at level (50 FVJ) may be attributed to (garlic and onion) extracts containing high quantities of bioactive substances; particularly organosulfur (allicin, allylmercaptan) and polyphenols compounds that have a tendency to reduce cellulolytic bacteria activity as observed by Prayitno *et al.*[32] and Oh *et al.*[33] who found that dairy cows administered with garlic extracts recorded the lowest values of CF and NDF digestibility as a result of the negative effects of garlic extract on some strains of cellulolytic bacteria. The results obtained from NDF and ADF digestibility can explained by Chesson *et al.*[34] who found that a plant containing polyphenols compounds has an inhibiting effect on some cellulolytic strains such as *Ruminococcus albus* and *Ruminococcus flavefaciens* whilst stimulate strains *Streptococcus bovis* and *Megasphaera elsdenii* that seemed to be more affected by cellulose degradation. These findings

of OM and CP digestibility reflected that the dosage at level 50 mL may be a more suitable concentration for rumen microbial activity in terms of cellulolytic and proteolytic organisms. That was proposed by Yang *et al.*[35] who found that the administering dairy cows with garlic oil increased ruminal Nitrogen digestibility that may be due to ability of proteolytic activity to take place in the rumen being stimulated. Also, Wanapat *et al.*[36], who case studies showed the positive impact of herb administration on OM digestibility. While, Miron *et al.*[37] found that the extract of herb plant additives to a cow's rations leads to an improvement on nutrient digestibility, except for fiber fraction digestibility.

The results of dry matter intake, suggested that the fruit and vegetable juice oral supplementation did not have an impact to the palatability for dairy cows. Similar results were shown by Oh *et al.*[33] who observed that dairy cows fed rations supplemented with the mixture of essential oil compounds or garlic oil recorded the same values of feed consumption.

Milk yield was ameliorating with animals undergoing oral administration (FVJ) especially at level 50 which may have various properties that were able to increase milk synthesis due to garlic and onion containing adequate amounts of selenium, chromium and sulfur.

These are considered an essential nutrient and their deficiency or excess for the optimal rate had been a negative impact on the rate of production[38-40]. Also, FVJ containing a sulfur-amino acid which has a positive effect on milk as a stimulator of milk protein synthesis and milk fat synthesis[41]. Although, decreased acetate levels were recorded in both levels of VFJ and increased propionate and butyrate, nevertheless level 50 VFJ recorded high milk fat (%), followed by level 100 VFJ that may be attributed to that butyrate is converted in the rumen to beta-hydroxybutyrate, which may contribute to the synthesis of milk fat with acetate which forms one half the synthesizing process of milk fat[42]. While, the other half of the fat is synthesized from fatty acids circulating in the blood cited by Ramos-Morales *et al.*[43] found that moderate increases in blood polyunsaturated fatty acids concentrations follow with a cow's oral supplementation of garlic oil.

Somatic cell count (SCC) was secreted in milk during a cows milking and is a general indicator of udder health and milk quality. Generally, when cows ranged an amount of SCC less than 200,000 cells mL^{-1} it indicates that animals are healthy and not infected with mastitis, reported by Schukken *et al.*[44]. In present experiment each cow recorded values less than 200,000 cells mL^{-1} and those results are compatible with Prayitno *et al.*[32], who showed that decreased levels of SCC in milk when cows supplemented by garlic extract. A possible interpretation of the effect of VFJ for decreased SCC was through previous research by Oh *et al.*[33] and Tuteja *et al.*[45] who reported that garlic and onion extract has an effective against mastitis bacteria.

Ruminal pH are consistent with decreased total VFA concentrations, as seen by the results obtained by this study and congruent with previous in vitro studies for cattle consuming diets which have shown the inclusion of allicin, allyl mercaptan and flavonoids having effects of reducing total VFA concentration and rising rumen pH levels by Kim *et al.*[46] and Oskoueian *et al.*[47]. NH_3-N concentration was decreased with increased doses of garlic powder in ruminant diets Kongmun *et al.*[48]. These results may be due to a close relationship between the decline in the percentage of ammonia and the ability of VFJ extracts on methane reduction in rumen thus reducing dehydrogenase activity[49], which leads to decreased NH_3-N in the rumen[50]. The results of the current study supported this hypothesis.

The decrease in total VFAs production and acetic proportion, increase of percentage of propionate and butyrate in present research results are confirmed by previous results obtained by Wanapat *et al.*[51] and Kongmun *et al.*[48], who showed that an increase of propionic (C3) and butyric (C4) for cow feed rations supplemented by garlic powder. Generally, NH_3-N concentration, total VFAs, acetate, total gas production and methane production decreased linearly with the increased dose of oral FVJ in comparison to the control group. The VFJ contains flavonoids, phenolic, mercaptan acid allicin and allyl which have the ability to modify rumen fermentation characteristics. Also, these changes are desirable in the rumen fermentation conditions like rise pH value, increase of propionate proportion, butyrate proportion, reduce protein degradation and reduce methane production[47]. Several previous studies concluded that the additive flavonoids to ruminant diets had beneficial effects on ruminal fermentation like reducing ammonia production caused by a decrease in protein degradation[52] and controlling rumen pH by the inhibition of lactate-producing bacteria i.e., *Lactobacillus* sp.[53] or stimulate lactate-consuming microorganisms i.e., *M. elsdenii*[54]. Gas production is *in vitro* as a rapid technique to evaluate the degradation of the material in the rumen. Decreased values of gas and methane production may be attributed to reduction of lactic acid bacteria that produce hydrogen and carbon dioxide as precursors for methanogens[55,56]. Furthermore, FVJ can be considered a source of polyphenols compounds and citrus fiber that reduce lactic acid bacteria counts[57]. Overall, Allium

family like onions (*Allium cepa*) or garlic (*Allium sativum*) are rich in phenolic compounds, which have effect against some strains of microbes in the rumen (*Methanobacterium formicicum*, *Methanobacterium bryantii*, *Mthanobrevibacter ruminantium*)[58,59], which reflected the reduction of total gas and methane production. The present results are in agreement with Busquet *et al.*[60] and Mirzaei-Aghsaghali *et al.*[61] who illustrated that the Allium family and its active components are effective against methanogenic bacteria.

The increased PCV, Hb and RBC content of the blood were an indication of the increasing capacity of the cells to carry oxygen, which is necessary for cell growth and energy. Consequently, this is reflected in the efficiency of animal health and growth[62]. The highest values of count WBCs are consistent with Oleforuh-Okoli *et al.*[62] and Eid and Iraqi[63], who reported that animals supplemented by garlic extract had the highest ($p<0.05$) counts of WBC compared with the control group. Leukocytes are the cells of the immune system that are involved in protecting the body against both infections and diseases. FVJ containing *Allicin* that has antibiotic effects[64] and containing vitamin C which production of lysozyme enzyme plays an important role in the prevention of bacterial infections[65], these substances are enhance the activity of phagocytic cells and lymphocytes that in effect increase WBC counts. The reduction in levels of AST and ALT may be attributed to allicin derivatives, which may protect the liver against deleterious agents and free radical-mediated toxic damage to the liver cells[66]. Furthermore, flavonoids have a protective effect on lipid metabolism, (So it reduces fat formation on the liver) oxidative stress and inflammation as well as their beneficial therapeutic effect of steatosis and liver inflammation. This is the scientific basis for the use of flavonoids in the treatment of non-alcoholic fatty liver disease (NAFLD)[67]. Even though FVJ mixtures extract contains vitamin C that have effects on the formation of oxalates in the kidneys[68], in spite of that serum total protein, urea and creatinine values were found within normal limits in the groups that were supplemented oral dose from FVJ that may be attributed to the existence of flavonoids that have beneficial effects for renal diseases such as glomerulonephritis, diabetic nephropathy and kidney insufficiency[69]. In this case prevention against any negative symptoms on the kidney can take place. The values of blood measures in the current study were within the normal range, which indicated no significant changes in animal's health related symptoms; this indicates that, the safety of using FVJ on ruminants.

CONCLUSION

Supplementation of FVJ (50 or 100 mL/cow/day) of dairy cow increases the feed digestibility, milk yield and milk fat. Moreover, FVJ contains active components that have modified rumen fermentation and could be of great interest for decreasing gas and methane production. Future research, including *in vivo* studies, in order to understand the factors that contribute to its antimicrobial activity and the selection of the optimal dose is required.

SIGNIFICANCE STATEMENT

This study discovers that supplementation of dairy cows with extract biological from fruit and vegetable juices that can be beneficial for improvement of feed digestibility, rumen fermentation, milk yield and milk fat. This study will help the researcher to uncover the critical areas of using several from extracts biological from fruit or vegetable as natural alternatives in animal ration for improve livestock productivity and their effects on animal health that many researchers were not able to explore. Thus a new theory on interest in the use of plants and their extracts or natural plant compounds in animal production and animal nutritionists as natural alternatives for improve feed efficiency and enhancing livestock productivity as replacement to antibiotics or synthetic compounds may be arrived at.

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