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TOP-DRESSING OF CHELATED PHYTOGENIC FEED ADDITIVES IN THE DIET OF LACTATING FRIESIAN COWS TO ENHANCE FEED UTILIZATION AND LACTATIONAL PERFORMANCE

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Abstract

The present experiment evaluated the inclusion of chelated phytogenic feed additives mixture in the diet of lactating cows for the first 3 months of lactation. A week before calving, thirty multiparous Friesian cows were divided into three treatments in a complete randomized design and fed a control diet without supplementation (control treatment), or the control diet supplemented with chelated phytogenic additives at 3 g (PHY3 treatment), or at 6 g/cow/d (PHY6 treatment). Menthol, levomenthol, β-linalool, anethole, hexadecanoic acid and P-menthane were the principal compounds identified in the additives mixture. Milk production, total solid, protein, fat, and lactose were increased with PHY3, but decreased by PHY6 (P<0.01). Whereas the PHY3 treatment increased (P<0.05) milk contents of Ca and Zn, PHY3 and PHY6 treatments increased (P<0.05) milk Fe and Mn concentrations. Though the PHY3 treatment increased (P<0.05) nutrient digestibility, the PHY6 treatment decreased (P<0.05) the digestibility of organic matter, crude protein and neutral detergent fiber. The PHY3 treatment increased (P<0.05) ruminal volatile fatty acids (VFA) concentration and proportional acetate and propionate and decreased butyrate, while the PHY6 treatment decreased ruminal VFA concentration and proportional acetate. The PHY3 treatment increased (P<0.05) serum total protein, glucose, total antioxidant capacity, and the concentrations of Ca and Zn. Both PHY3 and PHY6 treatment decreased (P<0.05) the concentrations of serum triglycerides, and cholesterol. Daily inclusion of 3 g/cow of chelated feed additives mixture in diet of lactating cows improved milk production and ruminal fermentation, but additives dose of 6 g/cow/d had negative impacts on cows' performance.

Key words: feed additives, phytogenic chelate, milk production, ruminal fermentation

Recently, there is increased interest in the use of natural and safe feed additives to replace ionophores and chemical feed additives to enhance lactational performance (Kholif et al., 2017 a, 2019) and health of animals (Rivero et al., 2016; Salem et al., 2017). Phytogenic feed additives such as herbal plants and seeds and essential

oils have been considered a new class of feed additives for livestock (Ebeid et al., 2020 b; Elghalid et al., 2020).

Combining phytobiotics can result in three different outcomes i.e., synergistic, additive, or antagonistic (Chouhan et al., 2017; Khattab et al., 2017). Synergy occurs when combining two additives produces antibacterial activity greater than the sum of the antibacterial activity of individual components (Chouhan et al., 2017) which has attracted an increased attention of animal nutritionists (Kholif et al., 2018 c, 2021; Elcoso et al., 2019). Benchaar et al. (2008) and Kholif et al. (2018 c) reported that feeding a combination of essential oils (capsicum and thymus) increased the antimicrobial activities and enhanced feed utilization and animal performance relative to individual ones. Antagonistic effects are other probable responses to combining phytogenics. The antagonistic effect decreases the antimicrobial activity of two compounds in combination as compared with their individual antimicrobial activity. There is a lack of detailed knowledge of the mechanism of the individual essential oil components which thus contributes to our superficial understanding of what governs synergy and antagonism (Chouhan et al., 2017; Kholif et al., 2017 b).

Balancing diet for minerals is essential for animal good performance and health (NRC, 2001; Bach et al., 2015; Kholif et al., 2020). Therefore, both health and productivity of dairy animals can be optimized through appropriate mineral nutrition (Bach et al., 2015; Kholif et al., 2020). Chelated minerals are preferred to minerals from inorganic sources to increase mineral bioavailability, absorption, and utilization and produce better results than traditional mineral supplementation (Nemec et al., 2012; Bach et al., 2015). Additionally, chelating decreases mineral excretion in feces and thus causes less environmental contamination (Flora and Pachauri, 2010). Trace minerals in chelated forms could supply an equivalent amount of mineral at lower dietary inclusion levels compared to inorganic forms (Cope et al., 2009).

The current study aimed to evaluate the effect of including two levels of phytogenic mixture chelate (a mixture of herbal plants, spices enriched with selected extracts and essential oils and minerals) in lactating Friesian cows' diet on feed consumption and efficiency, ruminal fermentation pattern, blood measurements, and milk yield and composition. It was hypothesized that biologically active components in the additives mixture and minerals would alter ruminal fermentation, affect nutrient digestion, and enhance milk production and its nutritive value.

Material and methods

Study location

The experiment was conducted at Noubaria Experimental Station, Animal Production Research Institute, Agriculture Research Centre and the laboratory of Dairy Animal Production, National Research Centre, Egypt. The farm is located at latitude 30°54'54.054" N and longitude 29°32'44.052" E. The farm had an annual average precipitation of 22 mm and average annual temperature of 14 to 32°C during experi-

ment. Animals care and handling were conducted under established approved standards of the Animal Production Research Institute, Egypt.

Chelated phytogenic mixture

A newly developed additives mixture (Aromix®, Masa Egypt Company, Alexandria, Egypt), comprising finely ground herbs and spices enriched with special extracts and essential oils as well as nonvolatile extracts, was evaluated in the diet of lactating cows. The volatile compounds in the additives mixture were determined using a Perkin Elmer Auto System XL GC/MS (Agilent, USA) and a capillary column ZB-5 (60 m × 0.32 mm i.d.; Agilent, USA), as described in details in Matloup et al. (2017).

For preparation of the phytogenic chelate, solutions of 0.08 mole of Ca-, Fe-, Mn-chloride, Cu-, and Zn-sulfates were mixed with additives mixture at 1:1. The reaction mixture was refluxed for two hours and then left overnight where the complexes were precipitated, filtered, washed with distilled water, and dried in vacuum desiccators over P_4O_{10}. The melting points of the complexes were over 300ºC (Cheong et al., 2012). For the analysis of the metal content, the complexes were digested and decomposed with aqua regia (a mixture of HCl acid and HNO_3 at a ratio of 3:1). The metal ion contents (Ca, Zn, Cu, Fe and Mn) were determined by atomic absorption spectra (Thermo Elemental, UK) in the Regional Centre for Food and Feed, Egypt. The laboratory is accredited according to ISO/IEC 17025 from A2LA. Results of atomic absorption showed that the chelated mixture contained (per kg): 28 g Ca, 25 g Cu, 16 g Fe, 15 g Mn and 15 g Zn. Concentrations of minerals in the chelated additives were formulated to cover the requirements of lactating cows according to NRC (2001).

Cows and management

Thirty multiparous lactating Friesian cows weighing 522 ± 9.8 kg with 7 ± 1 days in milk, 3 ± 1 parity and 13.0 ± 0.1 kg previous daily milk production were stratified randomly to three treatments (10 cows per treatment). Cows were assigned to the design sequentially in a period. Cows were fed a control diet (PHY0 treatment) containing [per kg dry matter (DM)] 500 g of concentrate feed mixture, 300 g of corn silage (*Zea mays*) and 200 g of rice straw (*Oryza sativa*) to meet their nutrient requirements according to NRC (2001) recommendations for first 3 months of lactation. In the other experimental diets, cows were supplemented with chelated additives mixture at 3 g (PHY3 treatment) or 6 g (PHY6 treatment)/cow daily based on the recent recommendations (Kholif et al., 2021). Adjustments were made to the amount of diets offered to ensure collection of leftovers (requirements + 10% margins). Diets were offered *ad libitum* to ensure collection of orts. Without any bedding, individual cows were housed in soil-surfaced tie stalls (122 × 175 cm^2/cow) under shades during feeding and sampling, while they were kept unrestrained in a barn during the rest of the time. Cows were fed daily at 07:00 and 21:00 h, with free access to water. The additives mixture was served once daily in 100 g concentrate DM to individual cows, prior to the morning feeding at 07:00 h to ensure that cows consumed the full dose. Daily samples of concentrate mixture, silage and rice straw

were collected, composited weekly, dried at 60°C in a forced-air oven for 48 h, and stored for chemical analyses. Ingredient and chemical compositions of the control diet are shown in Table 1.

Table 1. Chemical composition of ingredients and control diet fed to the cows (g/kg DM basis unless otherwise stated)

	Corn silage[1]	Rice straw	Concentrate feed mixture[2]	Control diet[3]
Dry matter (g/kg wet material)	302.4	895.4	898.3	719.0
Organic matter	937.3	905.9	950.2	937.5
Crude protein	79.4	33.7	161.4	111.3
Ether extract	26.2	10.2	31.3	25.6
Non-fibrous carbohydrates	406.8	111.5	420.4	354.5
Neutral detergent fiber	424.9	750.5	337.1	446.1
Acid detergent fiber	238.5	597.8	220.8	301.5
Digestible nutrients and energy value[4]				
Total digestible nutrients (g/kg DM)				436.8
Net energy of lactation (MJ/kg DM)				0.95

[1]Corn silage measurements: pH = 3.88; lactic acid concentration = 51.7 g/kg DM; acetate 14.7 g/kg DM; propionate = 5.9 g/kg DM.

[2]Consisted of (per kg DM): 405 g crushed corn grain, 255 g wheat bran, 175 g soybean, meal, 50 g undecorticated cottonseed meal, 50 g sugar beet pulp, 30 g molasses, 20 g limestone, 11 g sodium chloride, 4 g minerals and vitamins mixture [containing per kg: per kg: 141 g Ca, 87 g P, 45 g Mg, 14 g S, 120 g Na, 6 g K, 944 mg Fe, 1613 mg Zn, 484 mg Cu, 1748 mg Mn, 58 mg I, 51 mg Co, 13 mg Se, 24800 IU vitamin A, 7400 IU vitamin D3, 1656 IU vitamin E].

[3]The control diet based on (per kg DM): 500 g of concentrate feed mixture, 300 g of corn silage (*Zea mays*), and 200 g of rice straw (*Oryza sativa*).

[4]Calculated according to NRC (2001).

Feed intake and nutrient digestibility

Feed intake was recorded daily individually for each cow by weighing the offered diets and orts from the previous day. Using acid insoluble ash as an internal indigestible marker, nutrient digestibility trial was conducted at d 25 to d 31 and d 57 to d 63 (Sales and Janssens, 2003), while the apparent nutrient digestibility was calculated according to Ferret et al. (1999). Samples of fecal grab, collected twice daily at 07:00 and 15:00 h from 5 cows per treatment, were pooled per cow and dried at 60°C in a forced-air oven for 48 h.

Samples of feed, feed refusals, and feces were ground to pass a 1-mm screen using a Wiley mill and analyzed for DM (method 930.15), organic matter (OM; method 942.05), nitrogen (method 954.01), and ether extract (EE; method 920.39) according to AOAC (1997) official methods. Neutral detergent fiber (NDF) was determined by the procedure of Van Soest et al. (1991) with the use of an alpha amylase and sodium sulfite and expressed exclusive of residual ash. Acid detergent fiber (ADF) was analyzed according to AOAC (1997) (method 973.18) and expressed exclusive

of residual ash. Non-fibrous carbohydrate (1,000 – [NDF + crude protein (CP) + EE + ash]), and OM (1,000 – ash) were calculated.

Sampling and analysis of rumen fluid

On d 31 and d 63 of the assay, rumen contents were sampled at 3 h after the morning feeding to determine ammonia-N and total volatile fatty acids (VFA) concentrations. Using a stomach tube and pump, about 100 mL of rumen liquor was collected from the same cows used in the digestion trials and strained through 4 layers of cheesecloth. To avoid saliva contamination of ruminal content, the first 50 ml of the rumen fluid sample was discarded. A subsample of 5 mL of the strained rumen liquor was preserved in 5 mL of 0.2 *M* HCl for ammonia-N analysis (AOAC, 1997) (method 954.01) while another 0.8 mL was mixed with 0.2 mL of a solution containing 250 g of metaphosphoric acid/L for total VFA analysis by titration. Samples were stored at –20°C pending analyses. Concentration and molar proportions of individual VFA were determined using gas-liquid chromatography (model 5890, HP, Little Falls, DE, USA). 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 flame ionization detection.

Sampling and analysis of blood serum

On d 31 and d 63 of the assay, 10 mL of blood samples were taken 4 h after feeding from the jugular veins of the previously used 5 cows per treatment into clean dry anticoagulants free tube. Blood samples were centrifuged at 4,000 × *g* for 20 min. Serum was separated into 2 mL Eppendorf tubes and frozen at –20°C pending analysis. Using specific kits (Stanbio Laboratory, Boerne, Texas, USA) and following manufacturer instructions, selected serum parameters were determined. Selected serum minerals (Ca, Zn, Cu, Fe and Mn) concentrations were analyzed using atomic absorption spectrometry (Thermo Elemental, UK).

Milk sampling and composition

Cows were machine-milked, throughout the whole assay (9 weeks), twice daily at 06:00 and 18:00 h, and samples (100 g/kg 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 for the daily analysis. Milk constituents (total solids, fat, protein, and lactose) were analyzed using infrared spectrophotometry (Milkotester LM2, Belovo, Bulgaria). Milk minerals (Ca, Zn, Cu, Fe, and Mn) were analyzed using atomic absorption spectrometry (Thermo Elemental, UK). Energy-corrected milk (ECM) was calculated according to Sjaunja et al. (1991).

Statistical analyses

Data were analyzed as a completely randomized design with repeated measures using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC), with sampling time as repeated measures and individual animal as the experimental unit. Data for variables measured daily for each week (daily intake and milk production) were averaged before statistical analyses. The statistical model included the treatment ef-

fect, week, and treatment × week interaction. While treatment was the fixed effect, animal nested within treatment was the random effect. When the treatment *F*-test was significant at P<0.05, means were compared by applying the probability of difference option of the least squares means statement. Moreover, means were also compared using polynomial (curvilinear and quadratic) contrasts (adjusted for the equal spacing of treatments) to examine dose responses to increasing levels of the additives mixture. Significance was declared at P<0.05.

Results

Active compounds

A total of 19 peaks from the additives mixture extract were detected in the GC-MS chromatograms, with the retention time ranging between 6.73 and 35.73 min. Menthol, levomenthol, β-linalool, anethole, hexadecanoic acid and *p*-menthane were the principal compounds in the feed additives mixture (Table 2).

Table 2. Principal identified phytoconstituents of the additives mixture extract by GC-MS analysis[1]

Compound	RT	MW	Concentration
β-Linalool	6.73	154.1	124.0
p-Menthane	7.78	154.1	32.7
Menthol	7.95	156.2	133.0
Levomenthol	8.07	156.2	132.8
Anethole	10.31	148.1	116.7
Hexadecanoic acid	21.60	256.2	87.4

RT – retention time (min); MW – molecular weight of the compound; Concentration based on the total areas of the identified peaks (mg/g).

[1]Previously reported in Kholif et al. (2021).

Intake and lactational performance

Treatment did not affect intakes of corn silage, rice straw, or total feed intake (Table 3). The PHY3 treatment curvilinearly improved (P<0.01) daily milk production, while the PHY6 treatment decreased it compared with the control treatment (Table 3). Similarly, the PHY3 treatment curvilinearly improved (P<0.001) milk contents of total solid, and protein, and quadratically increased (P<0.001) fat and lactose but the PHY6 treatment decreased (P<0.001) milk contents of total solids relative to the control treatment. The PHY3 treatment increased milk contents of Ca (quadratic effect, P=0.022) and Zn (curvilinear effect, P<0.05), while both PHY3 and PHY6 treatments increased Fe (curvilinear effect, P<0.05) and Mn (curvilinear effect, P=0.011) concentrations, without affecting the concentrations of Cu compared with the control treatment. PHY treatments had no effect on milk efficiency calculated as milk/feed intake or ECM/feed intake.

Table 3. Intake and milk yield and composition of lactating Friesian cows fed a control diet supplemented with chelated additives mixture

	Treatments[1]			SEM	P value	
	PHY0	PHY3	PHY6		linear	quadratic
Intake (kg/cow/day)						
concentrate	5.84	5.84	5.84	0.000	1.000	1.000
corn silage	5.33	5.33	5.20	0.055	0.150	0.361
rice straw	2.25	2.13	2.13	0.051	0.116	0.388
total	13.4	13.3	13.2	0.07	0.430	0.930
Production (kg/d)						
milk	13.4 b	14.2 a	12.9 c	0.08	0.004	<0.001
energy corrected milk (ECM)	10.8 b	12.4 a	10.1 c	0.10	<0.001	<0.001
Milk composition (g/kg)						
total solids	105.2 b	113.2 a	102.1 c	0.45	<0.001	<0.001
protein	30.8 b	32.2 a	29.0 b	0.25	<0.001	<0.001
fat	30.5 b	33.4 a	29.9 b	0.34	0.217	<0.001
lactose	35.9 b	39.5 a	35.1 b	0.57	0.359	<0.001
Ca (mg/kg)	1376 b	1586 a	1475 ab	29.7	0.099	0.022
Zn (mg/kg)	3.50 b	4.05 a	3.81 b	0.065	0.042	0.016
Cu (mg/kg)	1.87	1.95	2.18	0.091	0.096	0.550
Fe (mg/kg)	10.4 b	11.5 a	11.5 a	0.11	0.006	0.032
Mn (mg/kg)	1.05 b	1.14 a	1.16 a	0.013	0.011	0.104
Milk (feed) efficiency						
milk/intake	1.00	1.07	1.03	0.031	0.488	0.185
ECM/intake	0.86	0.93	0.86	0.032	0.924	0.126

Means in the same row with different letters differ (P<0.05); SEM – standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g corn silage and 200 g rice straw without addition of supplements (PHY0 treatment) or with addition of 3 g chelated additives mixture (PHY3 treatment) or 6 g of chelated additives mixture/cow daily (PHY6 treatment).

Nutrient digestibility and ruminal fermentation

In contrast to the PHY3 treatment which increased DM (curvilinear effect, P<0.05), OM (curvilinear effect, P<0.001), CP (curvilinear effect, P=0.002), and NDF (curvilinear effect, P<0.05) digestibility, the PHY6 treatment decreased the digestibility of OM (curvilinear effect, P<0.001), CP (curvilinear effect, P=0.002), and NDF (curvilinear effect, P<0.05), without affecting the digestibility of EE or ADF (Table 4).

Treatments curvilinearly decreased (P=0.004) ruminal ammonia-N concentrations (Table 4). The PHY3 treatment curvilinearly decreased (P<0.05) butyrate proportion and increased ruminal VFA concentration (curvilinear effect, P<0.01), acetate (quadratic effect, P<0.05), and propionate (quadratic effect, P<0.05) proportions. The PHY6 treatment decreased ruminal VFA concentration (curvilinear effect, P<0.01) and proportion of acetate (quadratic effect, P<0.05), without affecting

ruminal propionate.

Table 4. Nutrient digestibility and ruminal fermentation of control diet supplemented with additives chelated mixture

	Treatments[1]			SEM	P value	
	PHY0	PHY3	PHY6		linear	quadratic
Digestibility (%)						
dry matter	62.63 b	66.71 a	60.06 b	0.318	0.029	0.005
organic matter	61.91 a	66.99 a	57.61 c	0.339	<0.001	<0.001
crude protein	60.61 a	57.70 b	54.77 c	0.643	0.002	0.995
ether extract	70.95	69.32	69.94	0.830	0.413	0.298
neutral detergent fiber	58.94 b	64.61 a	53.67 c	1.318	0.022	0.009
acid detergent fiber	49.59	53.05	48.67	1.131	0.581	0.221
Ruminal fermentation						
ammonia-N (mg/dL)	14.2 a	13.0 b	12.5 b	0.20	0.004	0.213
volatile fatty acids (mmol/L)	110.5 b	121.7 a	104.1 c	1.22	0.006	<0.001
acetate (mmol/100 mmol)	55.5 b	59.9 a	54.7 b	0.45	0.247	<0.001
propionate (mmol/100 mmol)	25.6 b	29.0 a	25.0 b	0.32	0.208	<0.001
butyrate (mmol/100 mmol)	18.9 a	11.1 b	20.3 a	0.443	0.036	0.022
acetate/propionate	2.17	2.13	2.23	0.042	0.346	0.203

Means in the same row with different letters differ (P<0.05). SEM – standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g corn silage and 200 g rice straw without addition of supplements (PHY0 treatment) or with addition of 3 g chelated additives mixture (PHY3 treatment) or 6 g of chelated additives mixture/cow daily (PHY6 treatment).

Blood measurements and antioxidant status

Treatments had no effect on blood concentrations of albumin, globulin, urea-N, creatinine, glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), malondialdehyde, catalase, glutathione peroxidase, superoxide dismutase, Cu, Fe, and Mn (Table 5). The PHY3 treatment increased serum total protein (quadratic effect, P=0.004), glucose (curvilinear effect, P=0.009), total antioxidant capacity (curvilinear effect, P<0.05), and the concentrations of Ca (curvilinear effect, P<0.05) and Zn (curvilinear effect, P=0.009). PHY treatments decreased (curvilinear effect, P<0.001) cholesterol compared with the control treatment.

Table 5. Blood chemistry (g/dL, unless stated otherwise) of lactating Friesian cows fed a control diet supplemented with chelated additives mixture

	Treatments[1]			SEM	P value	
	PHY0	PHY3	PHY6		linear	quadratic
1	2	3	4	5	6	7
Total proteins	7.51 b	7.87 a	7.34 b	0.063	0.095	0.004
Albumin	4.17	4.12	4.23	0.122	0.607	0.670
Globulin	3.37	3.75	3.11	0.141	0.227	0.018
Albumin/globulin ratio	1.23	1.10	1.41	0.091	0.204	0.084

Urea-N	22.8	21.7	22.8	0.37	0.940	0.041

Table 5 – contd.

1	2	3	4	5	6	7
Creatinine	0.85	0.88	0.88	0.008	0.330	0.258
Glucose	64.7 b	71.2 a	61.5 c	0.77	0.009	0.001
Glutamate-oxaloacetate transaminase (U/L)	37.2	35.9	36.6	0.35	0.203	0.052
Glutamate-pyruvate transaminase (U/L)	20.2	19.8	19.9	0.17	0.232	0.374
Triglycerides	93.4 a	69.7 b	69.1 b	1.01	<0.001	<0.001
Cholesterol	185.5 a	120.9 b	116.9 b	2.25	<0.001	<0.001
Malondialdehyde (µmol/mL)	27.5	25.4	26.1	1.41	0.508	0.462
Total antioxidant capacity (µmol/L)	88.7 b	99.5 a	96.3 a	2.30	0.046	0.039
Catalase (U/L)	5.47	5.91	5.75	0.166	0.274	0.181
Glutathione peroxidase (U/L)	2.79	3.00	2.99	0.232	0.563	0.705
Superoxide dismutase (U/L)	7.30	7.91	7.79	0.366	0.367	0.439
Ca (ppm)	51.8 b	60.9 a	60.4 a	0.82	0.005	0.018
Zn (ppm)	0.90 b	1.01 a	1.03 a	0.015	0.009	0.100
Cu (ppm)	0.74	0.81	0.85	0.091	0.454	0.901
Fe (ppm)	83.3	86.7	89.7	4.30	0.373	0.960
Mn (ppm)	0.14	0.14	0.15	0.019	0.742	1.000

Means in the same row with different letters differ (P<0.05). SEM – standard error of the mean.

[1]The control diet based on (per kg DM): 500 g of concentrates feed mixture, 300 g corn silage and 200 g rice straw without addition of supplements (PHY0 treatment) or with addition of 3 g chelated additives mixture (PHY3 treatment) or 6 g of chelated additives mixture/cow daily (PHY6 treatment).

Discussion

Feed intake and lactational performance

Generally, feed intake is affected by several factors, one is palatability; the others are digestibility, VFA production, rumen fermentation pattern, postruminal effects, metabolism, and other long term modulators. No difference in feed intake between treatments may indicate that the PHY mixture supplementation rates did not negatively affect palatability but differentially affected nutrient digestibility. In agreement, Elcoso et al. (2019) observed that feeding lactating cows on essential oils blend (eugenol, geranyl acetate, and coriander) did not affect feed intake. Additionally, Cortinhas et al. (2012) and Zhao et al. (2015) observed unaffected feed intake with feeding of chelated minerals (Zn, Cu and Mn) mixture to lactating Holstein cows.

The PHY3 treatment improved milk production and ECM by 6.4 and 14.9%, respectively, possibly caused by improved feed utilization and ruminal fermentation (Matloup et al., 2017; Kholif et al., 2018 b). In the present experiment, the PHY3 diet improved nutrient digestion and total ruminal VFA concentration which are the main reasons for the enhanced lactational performance (Morsy et al., 2018; Braun et al.,

2019; Elcoso et al., 2019). The greater milk yield with PHY3 could also be due to increased ruminal propionate which possibly increased lactose synthesis in mammary tissues. In agreement with our results, Santos et al. (2010) observed improved milk production with feeding essential oils complex, containing eugenol, geranyl acetate, and coriander oil as major components, to lactating cows.

It appears that the minerals (Zn, Mn, and Cu) in the chelated additives were involved in the improvement of the animals' physiological processes, resulting in increased milk production. Additionally, increased milk production with chelated minerals supplementation suggests improved efficiency of energy (NRC, 2001). del Valle et al. (2015) observed greater milk production and milk fat concentration when lactating cows were fed diet supplemented with organic minerals. In a meta-analysis study, Rabiee et al. (2010) demonstrated that supplementing diets of lactating animals with organic sources of minerals increased milk fat, protein, and yield. However, Cortinhas et al. (2012) and Zhao et al. (2015) reported no effects of chelated Zn or Cu on milk yield and composition. Variation between results of experiments may be due to the supplementation period, mineral source used, environmental conditions, and the requirements of animals.

The lowered milk production with the high level of the chelated phytogenic indicates negative effects of the additives on feed utilization and milk production. Parallel findings were reported by Malcolm-Callis et al. (2000).

Changes in feed digestion and ruminal fermentation directly affect milk composition (Linn, 1988; Yang and He, 2016). Improved OM digestibility resulted in increased ruminal propionate which could be a reason for the increased milk lactose with the PHY3 treatment (Kholif et al., 2019). Moreover, the PHY3 treatment increased milk fat content as a result of improved fiber digestion and ruminal acetate. Enhanced fiber digestion produces more ruminal acetate, the main precursor for the synthesis of milk fat (Linn, 1988; Santos et al., 2010). The above explanation becomes explicit and plausible when the results of PHY3 is compared with that of PHY6 which negatively affected fiber digestion and ruminal acetate and consequently decreased the milk fat concentration. Santos et al. (2010) and Kotsampasi et al. (2018) observed that orange peel essential oil supplementation increased milk fat concentration.

The PHY3 treatment increased milk contents of Ca and Zn. As previously noted, the PHY3 treatment increased milk protein which confirms the strong relationship between the concentrations of milk Ca and Zn and protein (Dunshea et al., 2019). Braun et al. (2019) observed that feeding essential oils containing menthol, as the major active compound, to lactating cows increased Ca levels in milk.

Nutrient digestibility and ruminal fermentation

The PHY3 treatment enhanced nutrient digestibility, while the PHY6 treatment lowered the digestibility of OM, CP, and NDF, indicating pronounced effect of the additives on ruminal microflora. Enhanced feed digestion without effect on intake reveals enhanced feed utilization. Patra et al. (2019) observed that supplementing diets of Suffolk sheep with menthol-rich plant bioactive lipid compounds at 80 and 160 mg/d increased the microbial phylogenetic diversity. Increased cellulolytic bac-

teria number with phytogenic feed additives (essential oils) was observed in some experiments (Giannenas et al., 2011; Kim et al., 2019). Kim et al. (2019) showed that essential oil mixture, containing eugenol, thymol, and cinnamaldehyde, increased the population of *Selenomonas ruminantium*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, fungi, and *Ruminococcus flavefaciens*. Additionally, the supplemental additives mixture contained menthol and anethole which possess dose-dependent antimicrobial activities against rumen protozoa and some gram-positive and gram-negative bacteria, but do not affect cellulolytic activity at doses within optimal range (Benchaar et al., 2008). This can explain the lowered nutrient digestibility with the high dose of the additives mixture. High additives doses can negatively affect feed digestion and fermentation due to their antimicrobial effects. Patra and Yu (2012) observed decreased population of archaea, protozoa, and major cellulolytic bacteria including *Fibrobacter succinogenes*, *R. flavefaciens,* and *R. albus* with increasing doses of clove oil, eucalyptus oil, garlic oil, origanum oil, and peppermint oil (0.10, 0.25, and 1.0 g/liter of *in vitro* fermentation medium).

The presence of the minerals mixture in the chelated additives may also be responsible for the improved nutrient digestibility (Durand and Kawashima, 1980). Mudita et al. (2014) showed that a mineral supplement caused increased rumen microbial protein synthesis. Wadhwa et al. (2016) reported that feeding chelated minerals increased microbial protein synthesis compared with inorganic minerals. del Valle et al. (2015) observed unaffected apparent nutrient digestibility with minerals supplementation.

Values of ruminal ammonia-N ranged between 12.5 and 14.2 mg/dL and were above the concentration (5 g ammonia-N/L) indicated for maximal microbial growth and activity (Satter and Slyter, 1974). PHY treatments decreased ruminal ammonia-N concentrations possibly as a result of lowered CP digestibility with the additives. These results reveal inhibited amino acids and protein deamination in the rumen (Benchaar et al., 2008) due to the phytogenic additives (e.g. thymol, eugenol, vanillin, guaiacol, and limonene) that have the ability to decrease the number of hyperammonia-producing bacteria (Giannenas et al., 2011).

The improved ruminal VFA concentration with PHY3 treatment could be the consequence of the enhanced nutrient digestibility (Pino and Heinrichs, 2016; Kholif et al., 2018 a). The concentrations of total VFA and their individual proportions solely depend on feed digestion and rumen microflora activity (Morsy et al., 2015; Ebeid et al., 2020 a). Additionally, the catalytic effect of trace minerals on enzymatic processes and structural and stabilizing functions on microorganisms can modify the metabolism of rumen bacteria and affect rumen fermentation (Durand and Kawashima, 1980). Matloup et al. (2017) and Morsy et al. (2018) reported greater VFA concentration with coriander oil and mustard and cumin seeds supplementation. Similarly, Pino and Heinrichs (2016) observed greater ruminal VFA concentration with feeding Cu, Mn, and Zn to dairy heifers.

In dairy production, increasing propionate is considered beneficial due to its effect on milk production. Phytogenic feed additives, such as essential oils (e.g. coriander oil, capsicum, and thymus), are well known to increase ruminal propionate (Matloup et al., 2017; Kholif et al., 2018 c) and decrease butyrate, as a result

of inhibiting *B. fibrisolvens* (Busquet et al., 2006). Busquet et al. (2006) reported a reduction in ruminal butyrate concentration with a number of essential oils. The PHY3 treatment increased ruminal propionate, revealing that ruminal fermentation became more gluconeogenic and improved energetic efficiency of the fermentation. Increased propionate could enhance the precursor's availability for lactose synthesis and promote nutrient utilization via the fermentation of sugars released by cell wall hydrolysis by ruminal enzymes (Linn, 1988).

Enhanced fiber digestion with the PHY3 treatment may be the main reason for the observed greater acetate proportion. Agarwal et al. (2009) observed that peppermint oil containing menthol, as a major component, increased ruminal acetate proportion. Pino and Heinrichs (2016) reported higher acetate molar proportion with no effect on propionate when trace minerals were fed to dairy heifer.

The decreased ruminal VFA concentrations and acetate proportion without any effect on ruminal propionate with PHY6 treatment confirm the negative effects of the high dose of phytogenic and minerals on ruminal fermentation.

Blood measurements and antioxidant status

The blood parameters were within optimal ranges for healthy cows (Etim et al., 2013), indicating safety of the evaluated chelated additives; however, the high additives dose negatively affected some of the measured blood parameters.

The PHY3 treatment increased serum total protein, indicating enhanced nutritional status of cows and minimal protein catabolism. Additionally, absence of significant effect of the treatments on serum urea-N and creatinine indicates normal kidney function. Cortinhas et al. (2012) observed that feeding Zn and Cu to lactating Holstein cows had no effect on the concentrations of serum total protein, albumin, and urea. The unchanged concentrations of GOT and GPT with increased total protein suggest absence of liver pathological lesions and normal liver activity and function (Pettersson et al., 2008), since liver plays an important role in protein metabolism and any damage in its cells is reflected in the total serum proteins and concentrations of GOT and GPT (Mbuh and Mbwaye, 2005; Pettersson et al., 2008).

The PHY3 treatment improved serum glucose level due to enhanced nutrient digestibility, indicating enhanced nutritional status of the cows. As previously noted, increased ruminal propionate with the additives could elevate precursor's availability for glucose and lactose synthesis (Linn, 1988). Kholif et al. (2021) observed similar results with feeding the same additives mixture without chelated minerals to lactating cows. However, Lakhani et al. (2019) observed that supplementing diets of growing buffalo calves with a mixture decreased serum glucose. Differences between studies may be due to different animal species, production type, age, and additives type.

The unchanged concentrations of malondialdehyde, catalase, glutathione peroxidase or superoxide dismutase reveal unaffected total antioxidant capacity with the phenolic compounds. However, Kotsampasi et al. (2018) observed that the phenolic compounds elicit antioxidant action, indicating improvement of animal health. Cortinhas et al. (2010) observed that feeding Zn, Cu, and Se to dairy cows had no effect on the serum superoxide dismutase or glutathione peroxidase concentration. Zhao

et al. (2015) noted that feeding chelated Zn, Cu, and Mn mixture to lactating cows increased serum Zn, Cu, and Mn, glutathione peroxidase and superoxide dismutase and decreased malondialdehyde without affecting catalase concentrations.

Additives at high or low levels of supplementation decreased the concentrations of cholesterol, suggesting a lowered atherogenic risk. Cortinhas et al. (2012) observed that feeding organic sources of Zn and Cu to lactating Holstein cows did not affect the concentrations of total cholesterol. Such results reveal that the lowered cholesterol, observed in the present study, could possibly be due to the phytogenic additives (e.g. ginger extract), but not the minerals in the additives mixture (Fuhrman et al., 2000). Polyphenolics and flavonoids in the additives mixture can inhibit the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, which is a key regulatory enzyme in the synthesis of cholesterol and lipids (Lee et al., 2004).

The PHY3 treatment increased serum Ca and Zn, revealing affected cation status in the cows and enhanced cation absorption (Braun et al., 2019). Kinal et al. (2007) observed increased Zn plasma in cattle supplemented with organic Zn. Increased Ca concentration in milk protects lactating cows from the metabolic disorder like milk fever (Martín-Tereso and Martens, 2014). In contrast to the present results, Cortinhas et al. (2012) observed that feeding organic or inorganic sources of Zn and Cu to lactating Holstein cows did not affect the concentrations of plasma Zn and Cu, glucose, urea, total cholesterol, total protein, albumin, GPT or GOT. The current assay suggests that the observed changes in blood chemistry were mainly due to the active components in the chelated additives.

Conclusions

The inclusion of chelated mineral containing phytogenic feed additives (ground herbs and spices enriched with selected and essential oils) at 3 g/cow daily in lactating cows diet improved feed digestion, ruminal fermentation and lactational performance. The 3 g/cow/d of chelated mineral containing phytogenic feed additives was the optimal dose, as the high dose (6 g/cow daily) had negative effects on feed utilization and milk production.

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