http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS
Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1380.1389

Research Article
Role of Caraway, Fennel and Melissa Addition on Productive Performance of Lactating Frisian Cows

Adel Eid Mohamed Mahmoud, Hassan Awny Fouad Rahmy and Wafaa Mostafa Ali Ghoneem

Department of Animal Production, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt

Abstract
Background and Objective: The use of aromatic and herbal plants as a feed additive became a new trend in feeding dairy cows to enhance animal productivity. This study was conducted to evaluate the effect of adding some herbal and aromatic plants (Melissa, Fennel and Caraway) at 0.7% of total DM of rations. **Materials and Methods:** Three herbal plants were evaluated in this study (melissa, fennel and caraway) by using thirteen Holstein Friesian cows. Cows were in 3rd and 5th lactation seasons were used as one experimental group by swing over design in lactation experiment (control then T_1 then T_2 then T_3 then control). Nutrients digestibility, milk production, milk composition and blood constituents were determined. **Results:** Data indicated that nutrients digestibility and nutritive value were improved with herbal plants addition, especially with caraway treatment which recorded significantly (p>0.05) the highest values. In the same trend, Actual milk yield, 4% FCM and fat content were significantly (p>0.05) increased when cows fed rations supplemented with fennel and caraway. The addition of melissa decreased the concentration of Total Saturated Fatty Acids (TSFA) in the milk. Normal ranges for blood parameters with no negative impact on animal health were observed with experimental rations. **Conclusion:** It could be concluded that caraway addition at 0.7% of the total dry matter of dairy rations, has a great potential to improve animal productivity and enhance the quality of milk fatty acids profile.

Key words: Melissa, Fennel, Caraway, lactating cows, milk yield, milk fatty acids profile

Citation: Adel Eid Mohamed Mahmoud, Hassan Awny Fouad Rahmy and Wafaa Mostafa Ali Ghoneem, 2020. Role of caraway, fennel and melissa addition on productive performance of lactating Frisian cows. Pak. J. Biol. Sci., 23: 1380-1389.

Corresponding Author: Adel Eid Mohamed Mahmoud, Department of Animal Production, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt Tel: 0020201117130183

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

Feed additives are used in animal feeding for different reasons. Some of these are to cover needs from essential nutrients and others like to increase feed intake, optimize feed utilization and improve overall animal performance. The 50-years practice of using antibiotics as feed additives for non-therapeutic purposes led to increased public and scientific concerns on their negative effects either on antibiotic resistance of human pathogenic bacteria[1], or the risk of passing their residues into animal products and consumers as subsequent. Therefore, the European Union introduced a general ban on the use of antibiotics as animal growth promoters[2]. Because of this restriction, consumers increasingly demanded more natural and safe products, which forcing producers to look for alternative feed additives. Natural products with a high concentration of secondary metabolites appear to be good as feed additives. The most important three types of feed additives in this category are probiotics, Enzymes and Herbs which have no residual effects.

Herbs can be used as a feed additive to promote the production industry and animal health. They may influence the feeding patterns, digestive fluids secretion and feed intake[3,4,5]. Also, they may be considered as growth promoters, which stimulate the endocrine system of the animal and enhance the metabolism of the nutrient by activating the enzymes concerned with the metabolism process[6,7]. The beneficial effects of using herbal plants in farm animals rations may be attributed to their positive effect on feed intake and activation of digestive enzymes secretion, or to their characteristics as antioxidative, antibacterial and immuno-stimulation[8,9].

Many substances found in herbs show prophylactic and therapeutic effects[10]. Aromatic plants, known as herbs and spices, are also used in medicine and food preservation. Polyphenolics are the main active compounds in aromatic plants, which have antimicrobial and antioxidant properties[11]. Recently, there is an interest in using bioactive plant factors that can modify microbial activities and metabolism in the rumen. The use of essential oils (plant extracts, phytofactors, volatile or ethereal oils) appears to be one of the most effective and natural alternatives instead of antibiotics in animal nutrition[12].

Rahmy *et al.*[13] concluded that the addition of 0.7% caraway, fennel or melissa has the potential to manipulate the *in vitro* rumen fermentation. It was observed that supplementation of dairy rations either with herbal plants[14,15,16] or their essential oils increased milk production[17,18]. Kolte *et al.*[19] showed that feeding lactating cows ration supplemented with herbal plants improved milk production and composition. Herbal plants had a positive impact on nutrients digestibility and performance[20,21]. Also, they can alter milk smell and flavor for consumers who exhibit on aversion to natural milk flavor[22].

Objectives of this study were to investigate the impact of incorporation three herbal and aromatic plants; melissa (*Melissa officinali*), fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*) at 0.7% of DM, as feed additives; in lactating Frisian cows ration on nutrients digestibility, nutritive values, milk production and composition and some blood parameters.

MATERIALS AND METHODS

This study was carried out at the Agriculture Experimental and Research Station, Animal Production Department, Faculty of Agriculture, Cairo University, Giza (September-December, 2017). The laboratory analyses were carried out at the labs of Faculty of Agriculture, Cairo University (January-April, 2018).

Herbal and aromatic plants: The herbal and aromatic plants were obtained from HARRAZ Company (Agricultural Seeds, Herbs and Plants Mixed spices, Bab ElKhalq Square, Cairo, Egypt).

Three species of herbal plants were used in this study (melissa, fennel and caraway). The percentages of essential oils (EO) and the bioactive compounds in the collected plant parts were determined as described by Lloyd and Miller[23]. It was shown from a previous study by Rahmy *et al.*[13] that the EO content in caraway was 0.54%. It contained 24.90% limonene, 0.21% trance di-hydrocarvone, 0.47% trance carveol, 74.17% carvone and 0.17% B-Caryophyllene. The EO content in fennel was 0.67%. It contained 0.31% α-pinene, 0.55% myrcene, 6.14% limonene, 2.55% 1,8 cineole, 88.9% methyl chavicol and 1.42% anethole. The EO content in melissa was 0.47%. It contained 0.95% b-pinene, 3.99% myrcene, 0.55% linalool, 32.02% neral (citral B), 53.74% geranial (citral A), 3.64% geranyl acetate and 2.38% B-caryophyllene.

Experimental animals and rations: The experiment was carried out on 13 Holstein-Friesian $\times$ Black and White Lowland crossbreed cows (in 3rd and 5th lactation seasons), lactation rank (2-4) and body weight (650$\pm$50 kg), at 80-days after calving. The experiment started and ended with a control ration without adding any herbal and aromatic plant as a feed additive. The animals were arranged in five swings over method design[24]. Swing over method is a design using in the lactation experiment through dealing with an animal as one group, start a trial and end experiment with control treatment;

Table 1: Chemical composition of the experimental rations (on DM basis)

	Experimental rations			
Item	C	T_1	T_2	T_3
DM	91.57	91.01	90.51	90.83
DM basis (%)				
OM	87.12	87.96	87.96	87.68
CP	16.32	16.53	16.74	17.07
CF	17.76	17.73	17.91	17.81
EE	3.00	4.47	3.76	4.34
NFE	50.04	49.23	49.55	48.46
Ash	12.88	12.04	12.04	12.32
Fiber fractions (%)				
NDF	34.34	36.62	37.21	36.33
ADF	25.22	25.57	25.80	24.99
ADL	6.65	6.82	7.01	6.63
Cellulose	18.57	18.75	18.79	18.36
Hemicellulose	9.12	11.05	11.41	11.34

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen free extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, C: Control "without supplement", T_1: Control with Melissa supplement, T_2: Control with Fennel supplement and T_3: Control with Caraway supplement

between two control treatments, other treatments put gradually. Swing over design aimed to use available capabilities and avoid the effects of female's physiological status and environmental effects. In that design through milk fat and milk production in two control, the researcher corrects the results of other treatments. The experiment continued for 90-days in 5-periods; each period consisted of 15-days for adaption and 3-days for samples collection.

Animals in the control group (C) were fed on alfalfa and rice straw plus concentrate feed mixture (CFM) (50% concentrate: 50% roughage), while animals in the tested groups (T_1, T_2 and T_3) were fed control ration plus 0.7% of melissa, fennel and caraway as feed additives, respectively. The ration was formulated to cover energy and protein requirements according to Paul *et al*.[25]. The basal ration consisted of 45% alfalfa hay, 5% rice straw, 25% yellow corn, 4% soybean meal, 10% wheat bran, 7.5% sunflower meal, 0.75% common salt, 1% limestone, 0.15% minerals and vitamins premix, 0.1% yeast, 0.7% sodium bicarbonate and 0.8% dicalcium phosphate. Chemical compositions of the experimental rations are shown in Table 1.

Feeding procedures: The CFM was offered to all animals after milking time (6 am and 6 pm). The restricted amounts of rice straw and alfalfa were offered for all animals. Clean water was available for animals all the time.

Milk collection, recording and sampling: Milk was obtained by milking unit housing system twice daily (6 am and 6 pm). The daily milk yield was individually recorded and was corrected to 4% FCM, using the following formula, according to Gains[26]:

$$\text{FCM (\%)} = 0.4\ (\text{milk yield}) + 15\ (\text{fat yield})$$

During the 3-days collection period, one tenth of the morning and evening milk yield were individually collected from each cow, then it mixed for each animal and stored at (-18°C) for further analysis.

Digestion trial: During the collection period, the digestion trial was carried out using all animals for three successive days. Digestion coefficients were determined by Acid insoluble ash (AIA) was used as an internal marker and calculated according to the following formula[27]:

$$\text{Digestion coefficient} = 100 - \left[100 \times \frac{\text{Indicator in feed (\%)}}{\text{Indicator in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in feed (\%)}}\right]$$

The nutritive values of the experimental rations as Total Digestible Nutrients (TDN) were calculated according to the classic formula of McDonald *et al*.[28] as follows:

$$\text{TDN (\%)} = [\text{digestible CP (\%)} + \text{Digestible CF (\%)} + \text{Digestible NFE (\%)} + (\text{Digestible EE (\%)} \times 2.25)]$$

Feces and blood sampling: Feces samples were withdrawn taken from the rectum by the end of each experimental period at 6 am, for three successive days from each animal. A subsample (10%) of each total collected feces of everyone was taken and sprayed with 10% formaldehyde and 10% sulfuric acid, respectively and then dried at 70°C for 24 h. Dried feces samples were kept individually in polyethylene bags for chemical analysis.

On the final day of the experimental period, blood samples were individually withdrawn from the jugular vein of individual animals. Blood samples were directly collected into clean dried heparinized glass tubes and centrifuged at 4000 rpm for 20 min. Blood plasma was then transferred into a clean dried glass vial then stored at -18°C until the chemical analysis.

Analytical methods: Chemical analysis of milk fat, protein, lactose and ash was determined by using Master Classic Ultrasonic Milk Analyzer. Milkotester Ltd. 49 Hristo Botev Str., 4470 Belovo, Bulgaria.

One gram from each milk sample to determine the fatty acids profile was dissolved in 10 mL hexane and 1 mL of sodium methoxide (5.4 M) in methanol solution. After strong vortexing for 1 min, 1 mL of the clear hexane layer was extracted by filtering through PTFE membrane, transferred into 2 mL GCMS vial from which 2 µL was injected for GCMS analysis; Instrument used: GC/MSD 5977A, Agilent, USA, Column used: Agilent, DB225 60 m×250 µm×0.25 µm. Oven program: 40°C for 1 min then 7.2°C/min to 195°C, 2.3°C/min to 230°C for 15 min Inlet: Split mode, split ratio 25:1 Liner Agilent 5190-2294: 990 µL Inlet Temperature: 250°C. Auxiliary temperature: 250°C.

Concentrate Feed Mixture (CFM), roughages and feces were analyzed for DM, ash, Crude Fiber (CF), Crude Protein (CP, Nitrogen×6.25) and Ether Extract (EE) contents according to AOAC[29]. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) contents were analyzed sequentially[30] using the ANKOM200. Cellulose and hemicellulose were calculated as following equations:

Cellulose = ADF-ADL and hemicellulose = NDF-ADF

Blood plasma constituents were determined using commercial kits. Total protein and creatinine were determined as described by Tietz[31,32], albumin was determined according to Doumas *et al.*[33], urea was determined according to Patton and Grouch[34]. Alanine aminotransferase (ALT) and activities of aminotransferase (AST) were determined according to Young[35].

Statistical analysis: Data were analyzed using the general linear model procedure of SAS[36]. The obtained data were analyzed using one-way analysis and according to the following model:

$$Y_{ij} = \mu + G_{ij} + E_{ij}$$

where, µ is the overall mean of Y_{ij}, G_{ij} is the group effect, E_{ij} is the experimental random error. The differences among means were separate according to Duncan's New Multiple Range test[37].

RESULTS AND DISCUSSION

Digestion coefficients and nutritive values of the experimental rations: It was observed from Table 2, that digestion coefficients of all nutrients and fiber fractions, except digestion coefficients of CP and EE were significantly ($p<0.05$) increased with herbal plants supplementation compared with control. While, there was no significant ($p<0.05$) difference between control and T_1 in the digestion coefficients of CP and EE. The highest nutrients digestibility was recorded with the caraway group (T_3) compared with other treatments.

Table 2: Digestibility and nutritive value of the experimental rations

	Experimental rations				
Item (%)	C	T_1	T_2	T_3	±SE
DM	62.79^d	70.04^b	66.74^c	73.92^a	0.636
OM	66.95^d	73.62^b	70.54^c	76.77^a	0.443
CP	75.62^c	76.19^c	78.10^b	83.94^a	0.358
CF	54.03^d	60.12^c	62.77^b	68.21^a	0.752
EE	71.01^c	70.88^c	77.19^b	81.82^a	0.727
NFE	55.26^d	58.65^c	62.06^b	70.03^a	0.812
NDF	50.02^c	59.56^b	59.74^b	66.60^a	0.924
ADF	42.64^c	51.92^b	51.94^b	63.35^a	0.984
Cellulose	68.24^b	75.09^a	69.79^b	75.95^a	0.900
Hemicellulose	70.44^c	77.69^a	77.42^a	73.80^b	0.490
Nutritive value					
TDN	54.38^c	59.26^b	61.60^b	68.40^a	1.701
DCP	12.34^b	12.59^b	13.07^{ab}	14.33^a	0.605

^{a,b,c,d}Means in the same row with different superscript are significantly different ($p<0.05$), DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen free extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, TDN: Total digestible nutrients, DCP: Digestible crude protein, C: Control "without supplement", T_1: Control with Melissa supplement, T_2: Control with Fennel supplement and T_3: Control with Caraway supplement

The improvement in nutrients digestibility may be attributed to the increase in CP contents in rations supplemented with the medicinal and aromatic plants, especially caraway. Also, the fiber structure of different herbal plants supplementation tended to be higher in hemicelluloses compared with control, indicating potential and good fermentable fibrous matter. In agreement with these results, Rahmy *et al.*[13] showed significant ($p<0.05$) increases *in vitro* DM, OM, NDF and ADF degradability when melissa, fennel and caraway added at 0.1, 0.3, 0.5 or 0.7% of total DM of ration.

Herbal plant extracts may improve nutrients digestibility due to their positive effects on total feed intake by add flavors to animal feeds, or by stimulating the digestion process through the digestive[5].

Different herbal plants such as *Trigonella foenum-graecum, Curcuma longum*, *Foeniculum vulgare*, *Allium sepa, Mentha piperita*, *Zingiber officinale* and *Cuminum cyminum* were found to improve the bile acids synthesis in the liver. Srinivasan[4] reported that most of the herbal plants and spices can stimulate secretion of digestive enzymes such as amylases, lipases and proteases. In addition, herbal plants and spices may reduce the passage rate of feed through the digestive tract[3,38,39,40]. Herbal plants have an antimicrobial activity which results in better utilization and absorption of nutrients or may result in activation of the immune system[3]. Herbal plants may stimulate the endocrine system and affect nutrients metabolism[6,7].

The nutritive value expressed as Total Digestible Nutrients (TDN) was significantly ($p<0.05$) improved with herbal and aromatic plants addition compared with control and the best significant ($p<0.05$) TDN was recorded with T_3 (68.40%) compared to 59.26 and 61.60%, respectively for T_1 and T_2 which were insignificantly ($p<0.05$) differ. Digestible crude protein (DCP) tended to increase significantly ($p<0.05$) with caraway treatment (T_3) being 14.32%, with no significant differences among T_1 (12.59%) and T_2 (13.07%) compared with control (12.34%).

The improvement in TDN and DCP values with caraway treatment (T_3) may be due to higher nutrients digestibility and CP content in T_3 compared with other treatments and control. Rahmy *et al.*[13] recorded lower *in vitro* ruminal ammonia concentrations with melissa, fennel and caraway supplementation, which revealed more CP degradability and the increase in microbial protein synthesis. The same authors reported significant ($p<0.05$) increases in ruminal VFA concentrations and total gas production when the pervious herbal plants added at 0.1, 0.3, 0.5 or 0.7% of the ration, which may be a good indicator of the improvement in energy and protein utilization with herbal plant supplementation[41].

Milk yield and composition: As shown in Table 3, there were significant ($p<0.05$) increases in average daily milk yield and 4% Fat Corrected Milk (FCM) with herbal additives compared with control.

The present results are reflections for the results of digestion coefficients and nutritive value, whereas caraway treatment (T_3) recorded the highest milk yield (21.90 kg/day) compared with other treatments and control. The increase in milk production with herbal plants supplementation may be attributed to their effect in enhancement feeds flavor and feed intake as consequent and to the improvement in nutrients digestibility as mentioned by Bhatt[3]. Also, Windisch *et al.*[5] showed that herbal and spices supplements have a positive effect on the health status of the animal and productivity by maintaining the stability of the gastrointestinal ecosystem through inhibition the growth of pathogens and reducing the animal exposure to toxins. These results are in agreement with the finding of Bhatt *et al.*[15] and Thakur *et al.*[42] that milk and FCM yields of lactating cows were significantly ($p<0.01$) improved with herbal supplementation. Also, Giannenas *et al.*[18] observed that the addition of essential oils at levels of 50, 100 and 150 mg kg^{-1} CFM, increased milk production of Chios dairy ewes. And Galbat *et al.*[16] recorded higher ($p<0.05$) milk yield when Egyptian dairy goats fed ration supplemented with a mixture of some medicinal plants (*Carum carvi, Trigonella foenum graecum* seeds*, Cuminum cyminum* and *Nigella sativa*). On the other hand, milk yield was not significantly ($p<0.05$) affected either when caraway seeds pulp replaced wheat bran by 33.3, 66.6 and 100% in rations of Holstein dairy cows[43], or when caraway and oregano EO were added to lactating cow rations at 0.2 and 1.0 g kg^{-1} DM[44]. Benchaar *et al.*[45,46] found that the addition of 750 mg or 2 g of MEO in rations of dairy cows, did not affect on milk production. Similar results had been recorded by Hosoda *et al.*[47], Yang *et al.*[48] and Benchaar *et al.*[49]. However, Russell *et al.*[50] recorded a significantly lower daily milk yield of lactating Sarda ewes fed *Melissia officinalis* ration.

Results showed that cows fed herbal rations recorded significant ($p<0.05$) increase in milk fat content compared with those fed control ration and T_2 recorded the highest significant ($p<0.05$) milk fat content being 4.36% compared with T_1 (3.88%), T_3 (3.94%) and C (3.53%).

The increase in milk fat content with herbal plants supplementation may be due to their higher oil contents, whereas, fennel had the highest oil content (0.67%) compared to 0.47 and 0.54% with melissa and caraway, respectively. The increase in fat synthesis with EO supplementation could be attributed to the increase in ruminal production of acetate compared with propionate[49,51]. In the same trend, Moheghi *et al.*[43] observed that milk fat content was increased linearly with increasing caraway level (3.3, 6.7 and 10%) in the ration of Holstein cows. Giannenas *et al.*[18] found that addition of EO preparation at 50, 100 and 150 mg kg^{-1} CFM, significantly ($p<0.05$) increased milk fat content. However, milk fat content was not significantly ($p<0.05$) affected either by addition of EO[44,49] or herbal and medicinal plants[16,42].

The present data indicated no significant effect of herbal plant supplementation on milk protein, lactose, ash and SNF contents. These results agree with those obtained by Giannenas *et al.*[18], Thakur *et al.*[42] and Moheghi *et al.*[43] who indicated that herbal plants or their EO had no significant

Table 3: Effect of experimental rations on milk yield and its composition

	Experimental rations				
Item	C	T_1	T_2	T_3	±SE
Milk yield (Kg/h/day)	18.80^{c}	20.04^{b}	21.60^{a}	21.90^{a}	0.256
FCM (Kg/h/day)	17.47^{d}	19.68^{c}	22.77^{a}	21.70^{b}	0.491
Fat (%)	3.53^{c}	3.88^{b}	4.36^{a}	3.94^{b}	0.141
Protein (%)	3.03	2.99	3.04	3.03	0.037
SNF (%)	8.24	8.13	8.25	8.67	0.299
TS (%)	11.77^{c}	12.01^{bc}	12.61^{a}	12.61^{a}	0.218
Lactose (%)	4.53	4.47	4.53	4.54	0.056
Ash (%)	0.68	0.67	0.68	0.69	0.031

[a, b, c, d] Means in the same row with different superscript are significantly different ($p<0.05$), C: Control "without supplement", T_1: Control with Melissa supplement, T_2: Control with Fennel supplement and T_3: Control with Caraway supplement, FCM: Fat corrected milk, SNF: Solid not fat and TS: Total Solid effect on milk composition. Also, Lejonklev *et al.* [44] observed no effect on milk protein content with caraway and oregano EO addition. While, milk protein and SNF contents were significantly ($p<0.05$) increased when dairy goats fed ration contained a mixture of medicinal plants[16]. Russell *et al.*[50] observed an insignificant ($p<0.05$) differ in milk lactose content and an increase in milk protein content with supplementation of melissa in lactating ewe's rations. On the opposite, Benchaar *et al.*[49] recorded higher milk lactose content when cows fed ration supplemented with essential oils compared with the control group, whereas milk protein content was not affected by EO addition. On the other hand, Malecky *et al.*[52] reported lower milk protein contents when a mixture of monoterpenes was added to the basal ration of Alpine and Saanen goats at 0.043 or 0.43 g kg^{-1} DM.

Data indicated that adding herbal plants in T_2 and T_3 rations significantly ($p<0.05$) increased TS content by 7% compared with control, while, the TS content was not significantly ($p<0.05$) differ between control and T_1. The increase in milk TS especially with T_2 and T_3 may be attributed to the increase in milk fat content. On the other hand, milk TS was not significantly ($p<0.05$) affected by caraway or a mixture of some medicinal plant's addition in lactating animals ration[16,43].

Milk fatty acids profile: The effect of herbal supplementation on milk fatty acid profile of lactating Frisian cows is shown in Table 4. Data showed that the short and medium chain fatty acids, including (C4:0. C6:0, C8:0, C9:0 and C12:0), were insignificant among treatments and control. While, the concentration of C10:0 was lower ($p<0.05$) in T_1 compared with other treatments and control. In the same trend, the concentrations of medium chain fatty acids including C14:0 and C15:0 were not significantly different among T_2, T_3 and control, however T_1 recorded the lowest C14:0 and the highest C15:0 concentrations. The effect of herbal plants supplementation on C16:0 was insignificant among groups, as well as C17:0 and C18:0, compared with the control group.

The lowest concentration of elaidic acid (C18:1 t-9) was observed with caraway treatment (T_3), with no significant difference compared with control. Many studies had evidence that high intake of trans-9 C18:1 (the trans-isomer of oleic acid; elaidic acid) has a negative impact on human health[53].

Data indicated that the addition of the tested herbal plants insignificantly increased the concentrations of omega 3 (α-linoleic acid; C18:3n-3) and poly-unsaturated fatty acids (PUFA). While, the concentrations of total saturated fatty acids (TSFA) were decreased especially with melissa treatment (T_1).

On the other hand, the addition of caraway EO at levels of 0.2 and 1.0 g kg^{-1} DM had no significant effect on concentrations of omega 3 in cows' milk[44]. The improvement in PUFA concentrations with herbal plant addition may be attributed to the antibacterial activity of EO against gram-positive bacteria[54], which participate in the biohydrogenation process of the unsaturated fatty acids the in rumen[55]. Benchaar *et al.*[46] reported no change in milk fatty acid profile when cows were supplemented daily with 750 mg of a mixture of essential oil compounds (MEO). However, supplementing the same MEO at a higher concentration (2 g day^{-1}) increased the concentration of Conjugated Linoleic Acid (CLA), a health-promoting fatty acid. Lourenço *et al.*[56] showed that the addition of 500 mg cinnamaldehyde/l in a dual-flow continuous bacterial culture fermentor, reduced the biohydrogenation process of polyunsaturated fatty acids.

Blood parameters as affected by the experimental rations: Data related to blood parameters are presented in Table 5. The

Table 4: Milk fatty acids profile as affected by herbal supplementation

	Experimental rations				
Item	C	T_1	T_2	T_3	±SE
C4:0, Butyric acid	4.180	3.500	3.030	5.220	0.431
C6:0, Caproic acid	2.680	1.470	1.330	3.150	0.340
C8:0, Caprylic acid	0.900	0.540	0.510	0.950	0.090
C9:0, Pelargonic acid	0.016	0.003	0.006	0.006	0.003
C10:0, Capric acid	1.800^{a}	1.270^{b}	1.340^{ab}	1.810^{a}	0.101
C12:0, Lauric acid	1.850	1.580	1.610	1.820	0.051
C14:0, Myristic acid	5.860^{a}	5.220^{b}	5.880^{a}	5.550^{ab}	0.102
C15:0, Pentadecylic acid	0.710^{b}	0.780^{a}	0.720^{b}	0.710^{b}	0.010
C16:0, Palmitic acid	57.410	59.930	59.750	56.840	0.634
C17:0, Margaric acid	0.120	0.330	0.320	0.320	0.038
C18:0, Stearic acid	5.770	5.140	5.650	4.840	0.193
C18:1t-9, Elaidic acid	17.000^{ab}	18.380^{a}	18.250^{ab}	16.840^{b}	0.278
C18:3n-3, Linolenic acid	1.320	1.620	1.640	1.670	0.083
C18:3, α-Linolenic acid	0.140	0.120	0.100	0.130	0.013
TSFA	81.300^{a}	79.760^{c}	80.150^{ab}	81.220^{a}	0.263
PUFA	1.460	1.740	1.740	1.800	0.076

[a, b, c] Means in the same row with different superscript are significantly different ($p<0.05$), C: Control "without supplement", T_1: Control with Melissa supplement, T_2: Control with Fennel supplement and T_3: Control with Caraway supplement, TSFA: Total saturated fatty acids, PUFA: Poly-unsaturated fatty acids

Table 5: Blood Parameters as affected by herbal supplementation

	Experimental rations				
Item	C	T_1	T_2	T_3	±SE
Total protein (g dL^{-1})	7.64^{b}	7.60^{b}	7.80^{a}	7.85^{a}	0.039
Albumin (g dL^{-1})	3.60	3.58	3.62	3.52	0.045
Globulin (g dL^{-1})	4.04	4.02	4.18	4.33	0.354
Urea (mg dL^{-1})	36.70^{a}	35.08^{b}	35.50^{b}	34.00^{c}	0.524
Creatinine (mg dL^{-1})	1.45^{a}	1.22^{b}	1.25^{b}	1.45^{a}	0.041
ALT (IU L^{-1})	35.50^{b}	34.41^{b}	36.33^{a}	34.33^{b}	0.325
AST (IU L^{-1})	34.79	34.25	34.41	34.16	0.488

[a, b, c] Means in the same row with different superscript are significantly different ($p<0.05$), C: Control "without supplement", T_1: Control with Melissa supplement, T_2: Control with Fennel supplement and T_3: Control with Caraway supplement

average values of total protein were significantly ($p<0.05$) increased with T_2 T_3 and it insignificantly ($p<0.05$) decreased with T_1 compared with control.

The increase in blood TP especially with caraway addition may be due to the increase in DCP with T_3 being 14.32% compared with other treatments and control. Same result with observed by Galbat *et al.*[16] that the concentration of serum TP was increased with the addition of some medicinal plants containing caraway (*Carum carvi*) in the ration of dairy goats.

There were insignificant ($p<0.05$) differences among groups in the contents of albumin and globulin. The highest concentration of blood globulin was recorded for T_3 (4.33 g dL^{-1}) and the lowest value was recorded for T_1 (4.02).

The increase in blood globulin with herbal plants supplementation may be evidence of their ability to improve animal immunity because of inhibition of pathogens growth or reducing their toxins into the digestive tract[5]. Also, Bhatt[3] reported that most herbal plants have antibacterial, anti-viral, anthelmintic, anti-inflammatory, coccidiostats or antioxidant properties, which may enhance the immune system. Results have coincided with those obtained by Kumar *et al.*[57] who reported significant increases in concentrations of total serum protein and globulin when *Sesbania grandiflora* was supplemented in cows at 0 to 5 g/head/day for 45 days.

The concentration of blood urea was significantly ($p<0.05$) decreased with cows fed herbal plant rations compared with control and the lowest concentration was recorded with caraway ration (T_3).

The decrease in blood urea concentrations with herbal plants addition may be due to the improvement in microbial protein synthesis in the rumen. In agreement with this explanation, *in vitro* concentrations of ruminal ammonia were decreased when ration supplemented with caraway, fennel or melissa at levels[13] of 0.1, 0.3, 0.5 or 0.7%. In the same context, Lewis[58], Petit and Flipot[59] and Davidson *et al.*[60] showed a positive correlation between concentrations of ruminal ammonia and urea nitrogen in blood (BUN). Wallace *et al.*[61] showed that EO could inhibit the attachment of ruminal bacteria to feed particles, which decreases AA deamination and ammonia production as subsequently. This reduction in ruminal ammonia may be attributed to the negative effect of EO supplementation on the type and number of hyper ammonia producing[62]. Both reduction in ruminal ammonia nitrogen and Blood Urea Nitrogen (BUN) may provide evidence for ruminal bypass protein[63,64,65]. In the same trend, Moheghi *et al.*[43] observed linear decreases in concentrations of serum urea N with increasing level of caraway seeds in the rations (3.3, 6.7 and 10%). Wanapat *et al.*[66] reported significant ($p<0.05$) decreases in BUN concentrations with herbal plants supplementation. Galbat *et al.*[16] found that supplementation of dairy goat's ration with a mixture of some medicinal plants containing caraway (*Carum carvi*) decreased concentration of serum urea.

No significant differences were observed in concentrations of blood creatinine between T_3 and control, however T_1 and T_2 groups significantly ($p<0.05$) decreased compared to control. Blood ALT concentrations were insignificantly ($p<0.05$) differ among C, T_1 and T_3, while it was significantly ($p<0.05$) increased with T_2 compared with other feeding groups. On the other hand, concentrations of blood AST were not significantly ($p<0.05$) changed by herbal plants addition compared with control.

In agreement with our results, Galbat *et al.*[16] recorded an insignificant decrease in concentrations of serum ALT and AST when a mixture of some medicinal plants containing caraway (*Carum carvi*) added to the ration of dairy goats. Also, Hajalizadeh *et al.*[67] recorded insignificant differences in blood AST and ALT concentrations between lambs fed ration supplemented with 1.5% fennel and those fed control. However, Moheghi *et al.*[43] observed linear increases in concentrations of serum AST and ALT with an increasing level of caraway seeds in the rations (3.3, 6.7 and 10%).

The present results indicated no adverse effects on tested blood parameters of lactating cows due to herbal addition to the ration, since these values of blood parameters were all within the normal physiological ranges, as reported by Kaneko *et al.*[68].

Generally, the results of this study cleared that the addition of an active part (contains an active component) of herbal plants as it could enhance the performance of lactating cows, especially the addition process was easily more than the extracts of those plants. Also, the results of the milk fatty acids profile explained that active components in the aromatic and herbal plants have a positive effect on the biohydrogenation process in the rumen. So, more studies needed to investigate the effects of aromatic plants in rumen ecology. Also, more research needed to examine more and new aromatic and herbal plants.

CONCLUSION

It could be summarized that the addition of 0.7% of Melissa, fennel and caraway in lactating Frisian cow's ration increased nutrients digestibility, nutritive values, milk production and milk fat content. Moreover, concentrations of α-linoleic acid (omega 3) and PUFA were increased. Blood parameter results referred to there were the positive effect of herbal plants on animal health.

SIGNIFICANT STATEMENT

Findings in this study investigated that the addition of herbal plants in diets could increase the productivity of dairy cows and improve milk quality. Where results referred to fennel increase the milk production by 30% and milk fat by 23% compared with the control group. Also, the addition of caraway increased the percentage of polyunsaturated fatty acids (PUFA) by 26.51% and α-linoleic acid (C18:3n3) by 23.28% compared with the control diet. This study will help the researchers to uncover the different effects of using herbal plants. Thus, a new theory in the applications of herbal and aromatic plants in animal feeding as phytogenic feed additives.

REFERENCES

1. Barton, M.D., 2000. Antibiotic use in animal feed and its impact on human health. Nutr. Res. Rev., 13: 279-299.
2. European Union, 2003. Regulation (EC) no.1831/2003 of the European parliament and of the council of 22 September 2003. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32003R1831.
3. Bhatt, N., 2015. Herbs and herbal supplements, a novel nutritional approach in animal nutrition. Iranian J. Appl. Anim. Sci., 5: 497-516.
4. Srinivasan, K., 2005. Spices as influencers of body metabolism: An overview of three decades of research. Food Res. Int., 38: 77-86.
5. Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr, 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci., 86: E140-E148.
6. Camara, C.C., N.R. Nascimento, C.L. Macedo-Filho, F.B.S. Almedia and M.C. Fonteles, 2003. Antispasmodic effect of the essential oil of *Plectranthus barbatus* and some major constituents on the guinea-pig ileum. Planta Med., 69: 1080-1085.
7. Madeira, S.V.F., F.J.A. Matos and D.C. Leal-Criddle, 2002. Relaxant effects of the essential oil of *Ocimum gratissimum* on isolated ileum of the guinea pig. J. Ethnopharmacol., 81: 1-4.
8. Halliwell, B., R. Aeschbach, J. Lo Liger and O.I. Aruoma, 1995. The characterization of antioxidant. Food Chem. Toxicol., 33: 601-607.
9. Uegaki, R., S. Ando, M. Ishida, O. Takada, K. Shinokura and Y. Kohchi, 2001. Antioxidative activity of milk from cows fed herbs. J. Agric. Chem. Soc. Japan, 75: 669-671.
10. Linde, K., A. Vickers, M. Hondras, G.T. Riet, J. Thormählen, B. Berman and D. Melchart, 2001. Systematic reviews of complementary therapies - an annotated bibliography. Part 1: Acupuncture. BMC Complement. Altern. Med., 10.1186/1472-6882-1-3
11. Christaki, E., E. Bonos, I. Giannenas and P. Florou-Paneri, 2012. Aromatic plants as a source of bioactive compounds. Agriculture, 2: 228-243.
12. Mazaher, H., 2014. The effects of use medicinal plants on rumen fermentation parameters in ruminants. Int. J. Adv. Bio. Biomed. Res., 4: 1318-1327.
13. Rahmy, H.A.F., H.M. El Bana, N.E. El-Bordeny, A.E.M. Mahmoud and W.M.A. Ghoneem, 2019. Effect of caraway, fennel and melissa addition on *in vitro* rumen fermentation and gas production. Pak. J. Biol. Sci., 22: 67-72.
14. Abo El-Nor, S.A.H., H.M. Khattab, H.A. Al-Alamy, F.A. Salem and M.M. Abdou, 2007. Effect of medicinal plant seeds in the rations on the productive performance of lactating buffaloes. Int. J. Dairy Sci., 2: 348-355.
15. Bhatt, N., M. Singh and A. Ali, 2009. Effect of feeding herbal preparations on milk yield and rumen parameters in lactating crossbred cows. Int. J. Agric. Biol., 118: 721-726.
16. Galbat S. A., A. El-Shemy, A.M. Madpoli, O.M.A.L. Maghraby and E.I. El-Mossalami, 2014. Effects of some medicinal plants mixture on milk performance and blood components of Egyptian dairy goats. Middle East J. Appl. Sci., 4: 942-948.
17. Benchaar, C., S. Calsamiglia, A.V. Chaves, G.R. Fraser, D. Colombatto, T.A. McAllister and K.A. Beauchemin, 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed. Sci. Technol., 145: 209-228.
18. Giannenas, I., J. Skoufos, C. Giannakopoulos, M. Wiemann, O. Gortzi, S. Lalas and I. Kyriazakis, 2011. Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes. J. Dairy Sci., 94:5569–5577.
19. Kolte, A.Y., S.P. Waghmare, S.G. Mode and A. Handa, 2008. Efficacy of indigenous herbal preparation on altered milk pH, somatic cell count and electrolyte profile in subclinical mastitis in cows. Vet. World, 1: 239-240.
20. Broudiscou, L.P., Y. Papon and A.F. Broudiscou, 2002. Effects of dry plant extracts on feed degradation and the production of rumen microbial biomass in a dual outflow fermenter. Anim. Feed Sci. Technol., 101: 183-189.
21. Tedesco, D., A. Tava, S. Galletti, M. Tameni, G. Varisco, A. Costa and S. Steidler, 2004. Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. J. Dairy Sci., 87: 2239-2247.
22. Ando, S., T. Nishida. M. Ishida, Y. Kochi, A. Kami and S. Se, 2001. Transmission of herb essential oil to milk and change of milk flavor by feeding dried herbs to lactating holstein cows. J. Japanese Soc. Food Sci. Technol., 48: 142-145.
23. Lloyd, C. and P.D. Miller, 1963. pharmacopoeia and formularies. The-British-pharmacopoeia. https://doi.org/10.1111/j.2042-7158.1963. tb12872.x.
24. El-Serafy, A.M., 1968. Some nutritional studies on the suitable combination of molasses with certain feedstuffs. M.Sc. Thesis, Cairo University, Egypt.
25. Paul, S.S., A.B. Mandal and N.N. Pathak, 2002. Feeding standards for lactating riverine buffaloes in tropical conditions. J. Dairy Res., 69: 173-180.

26. Gaines, W.L., 1928. The energy basis of measuring milk yield in dairy cows. MSc Thesis, University of Illinois Agricultural Experiment Station, Urbana-Champaign Campus, Chicago, Illinois.
27. Van Keulen, J. and B.A. Young, 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci., 44: 282-287.
28. McDonald, P., R.A. Edwards, C.A. Morgan and J.F.D. Greenhalgh, 1995. Animal Nutrition. 5th Edn., Pearson Education Limited, Harlow, United Kingdom, Pages: 624.
29. AOAC, 2000. Official methods of analysis. 17th Edn. Association of Official Analytical Chemists, Washington, DC, USA.
30. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
31. Tietz, N.M., 1986. Textbook of Clinical Chemistry. 3rd Edn., W. B. Saunders Company, Philadelphia, London, Toronto, pp: 510.
32. Tietz, N.W., 1990. Clinical Guide to Laboratory Tests. 2nd Edn., W.B. Saunders Company, Philadelphia, PA., USA., ISBN-13: 978-0721624860, pp: 554-556.
33. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.
34. Patton, C.J. and S.R. Crouch, 1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464-469.
35. Young, D.S., 1990. Effects of Drugs on Clinical Laboratory Tests. 3rd Edn., AACC Press, Washington, DC.
36. SAS, 2012. SAS users guide statistical analysis system institute. Inc., Cary, Nc, USA.
37. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
38. Frankic, T., M. Voljc, J. Salobir and V. Rezar, 2009. Use of herbs and spices and their extracts in animal nutrition. Acta Agriculturae Slovenica, 94: 95-102.
39. Platel, K. and K. Srinivasan, 2001. Studies on the influence of dietary spices on food transit time in experimental rats. Nutr. Res., 21: 1309-1314.
40. Suresh, D. and K. Srinivasan, 2007. Studies on the *in vitro* absorption of spice principles-curcumin, capsaicin and piperine in rat intestines. Food Chem. Toxicol., 45: 1437-1442.
41. Cedillo, J, J. F. Vázquez-Armijo, A. González-Reyna, A.Z.M. Salem, A.E. Kholif, J. Hernández-Meléndez, J.C. Martínez-González, R.M.O. Jiménez, N. Rivero and D. López, 2014. Effects of different doses of Salix babylonica extract on growth performance and diet *in vitro* gas production in Pelibuey growing lambs. Italian J. Anim. Sci., 13:609-613.
42. Thakur, S.S., A.K. Tyagi and K.K. Singhal, 2006. Effect of a commercial herbal feed supplement on the performance of lactating cows. Indian J. Anim. Nutr., 23: 244-246.
43. Moheghi, M.M., A.M. Tahmasbi, A.A. Naserian and A. Omid, 2010. Assessment of nutritional value of caraway-seed pulp for feeding Holstein dairy cattle. J. Anim. Vet. Adv., 9: 306-310.
44. Lejonklev, J., U. Kidmose, S. Jensen, M.A. Petersen and A.L.F. Helwing *et al*., 2016. *Short communication*: Effect of oregano and caraway essential oils on the production and flavor of cow milk. J. Dairy Sci., 99: 7898-7903.
45. Benchaar, C., J.L. Duynisveld and E. Charmley, 2006. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can. J. Anim. Sci., 86: 91-96.
46. Benchaar, C., A.V. Chaves, G.R. Fraser, Y. Yang, K.A. Beauchemin and T.A. McAllister, 2007. Effects of essential oils and their components on *in vitro* rumen microbial fermentation. Can. J. Anim. Sci., 87: 413-419.
47. Hosoda, K., T. Nishida, W.Y. Park and B. Eruden, 2005. Influence of mentha×piperita L. (Peppermint) supplementation on nutrient digestibility and energy metabolism in lactating dairy cows. Asian Australas. J. Anim. Sci., 18: 1721-1726.
48. Yang, W.Z., A.V. Chaves, M.L. He, C. Benchaar and T.A. McAllister, 2006. Abstracts presented at the joint CSA, CSHS and CSAS meeting. Can. J. Anim. Sci., 86: 577-600.
49. Benchaar, C., H.V. Petit, R. Berthiaume, D.R. Ouellet, J. Chiquette and P.V. Chouinard, 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci., 90: 886-897.
50. Russell, J.B., H.J. Strobel and G.J. Chen, 1988. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Applied Environ. Microbiol., 54: 872-877.
51. Agarwal, N., C. Shekhar, R. Kumar, L.C. Chaudhary and D.N. Kamra, 2009. Effect of peppermint (*Mentha piperita*) oil on *In vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Anim. Feed Sci. Technol., 148: 321-327.
52. Malecky, M., L.P. Broudiscou and P. Schmidely, 2009. Effects of two levels of monoterpene blend on rumen fermentation, terpene and nutrient flows in the duodenum and milk production in dairy goats. Anim. Feed Sci. Technol., 154: 24-35.
53. Kuhnt, K., M. Baehr, C. Rohrer and G. Jahreis, 2011. Trans fatty acid isomers and the trans-9/trans-11 index in fat containing foods. Eur. J. Lipid Sci. Technol., 113: 1281-1292.
54. Helander, I.M., H.L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm and I. Pol *et al*., 1998. Characterization of the action of selected essential oil components on gram-negative bacteria. J. Agric. Food Chem., 46: 3590-3595.
55. Harfoot, C.G. and G.P. Hazlewood, 1988. Lipid Metabolism in the Rumen. In: The Rumen Microbial Ecosystem, Hobson, P.N. (Ed.). Elsevier, London, UK., pp: 285-322.

56. Lourenco, M., P.W. Cardozo, S. Calsamiglia and V. Fievez, 2008. Effects of saponins, quercetin, eugenol and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. J. Anim. Sci., 86: 3045-3053.
57. Kumar, A., N. Gupta and D.P. Tiwari, 2006. Effect of herbs as feed additive on *In vitro* and in sacco dry matter digestibility of paddy straw. Indian J. Anim. Sci., 76: 847-850.
58. Lewis, D., 1957. Blood urea concentration in relation to protein utilization in ruminants. J. Agric. Sci., 48: 438-466.
59. Petit, H.V. and P.M. Flipot, 1992. Feed utilization of beef steers fed grass as hay or silage with or without nitrogen supplementation. J. Anim. Sci., 70: 876-883.
60. Davidson, S., B.A. Hopkins, D.E. Diaz, S.M. Bolt, C. Brownie, V. Fellner and L.W. Whitlow, 2003. Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization and excretion in early lactation holstein cows. J. Dairy Sci., 86: 1681-1689.
61. Wallace, R.J., N.R. McEwan, F.M. McInotoch, B. Teferedegne and C.J. Newbold, 2002. Natural products as manipulators of rumen fermentation. Asian-Australasian J. Anim. Sci., 10: 1458-1468.
62. McEwan, N.R., R.C. Graham, R.J. Wallace, R. Losa, P. Williams and C.J. Newbold, 2002. Effect of essential oils on ammonia production by rumen microbes. Reprod. Nutr. Dev., 42: S65-S65.
63. Cardozo, P.W., S. Calsamiglia, A. Ferret and C. Kamel, 2005. Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle. J. Dairy Sci., 83: 2572-2579.
64. Newbold, C.J., F.M. McIntosh, P. Williams, R. Losa and R.J. Wallace, 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 114: 105-112.
65. Wallace, R. J., L. Athaud and C.J. Newbold, 1994. Influence of Yucca shidigera extract on rumen ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol., 60: 1726-1767.
66. Wanapat, M., S. Kang, P. Khejornsart and S. Wanapat, 2013. Effects of plant herb combination supplementation on rumen fermentation and nutrient digestibility in beef cattle. Asian-Australasian J. Anim. Sci., 8: 1127-1136.
67. Hajalizadeh, Z., O. Dayani, A. Khezri, R. Tahmasbi and M.R. Mohammadabadi, 2019. The effect of adding fennel (*Foeniculum vulgare*) seed powder to the diet of fattening lambs on performance, carcass characteristics and liver enzymes. Small Ruminant Res., 175: 72-77.
68. Kaneko, J.J., J.W. Harvey and M.L. Bruss, 1997. Clinical Biochemistry of Domestic Animals. 5th Edn., Academic Press, San Diego, California, ISBN-13: 9780123963055, Pages: 932.