Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 3 ~ Issue 11 (2016) pp: 01-06
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Effect of Tea Leaf and Soybean Oil Supplementation on Milk Composition of Dairy Cows

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Received 19 April, 2016; **A**ccepted 10 May, 2016 © The author(s) 2016. Published with open access at **www.questjournals.org**

ABSTRACT:- *This research studied the effect of tea leaf - Camellia sinensis (TL) and soybean oil (SBO) supplementation on milk composition of low yield dairy cows in a tropical climate. These supplementations were chosen because they can aid in controlling CH_4 and CO_2 emissions. Three ruminally cannulated dairy cows during their first lactation were used in an experiment with 3 × 3 Latin square design. Tests were done by adding the supplementations to the total mixed ration fed to the cows during 7 d. Results show that SBO supplementation increased milk fat percentage without affecting milk yield or protein, lactose or solid non fat content in milk. The acetate to propionate ratio was lower after SBO supplementation since, under heat stress, an extra boost of energy may have greater effect over milk fat production than the volatile fatty acid profile. On the other hand, a positive effect of TL supplementation on milk yield was found, due to the caffeine content in the tea and an increase in rumen butyrate and propionate. These results suggest that SBO supplementation is an alternative for solving a negative energy balance in Holstein cows due to heat stress in tropical countries, while also decreasing greenhouse gas emissions.*

Keywords: *dairy cows, milk quality, soybean oil, tea leaves (Camellia sinensis).*

I. INTRODUCTION

Bovine milk composition can be changed via nutritional control [1]. The composition of milk is influenced by factors such as management, nutrition, age, stage of lactation, breed and genetics [2,3]. A nutrient must first be incorporated into the diet of the cow, absorbed into the blood stream and transported to the mammary gland, where it ends up being secreted as a component in milk or functioning as a regulator of milk synthesis [1]. The ability to manipulate fat and protein proportions is important because consumer preferences for these may change rapidly [4]. Lactose, on the other hand, is not expected to be manipulated via dietary change [1].

Fat is the component of milk that is the most sensitive to dietary change; it can be changed over a range of 3 percentage units [1]. Manipulating the diet will affect both fat content and milk fat yield [4]. Milk composition has been manipulated via oil supplementation, for example oilseeds [5] soybean oil [6] and palm oil [7]. Fat supplementation can have three advantages: 1) increase in the energy density of the diet; 2) increase in energetic efficiency due to reduced heat loss and methane (CH_4) and urine production; and 3) risk reduction of rumen acidosis [8]. Nonetheless, feeding supplemental fat has been shown to cause depression in fat and protein concentration in milk due to its effect on 1) feed intake and 2) digestion of fiber in the rumen [8]. Therefore, it is important to consider the fat amount and source to understand how adding fat to the ration may affect milk component levels. Polyunsaturated fat (i.e., vegetable oil) supplementation has been shown to reduce milk fat because it reduces digestibility of fiber and the acetate to propionate ratio. Nonetheless, when fat is increased in the diet in an effort to reduce dietary starch, the effect may be an increase in the acetate to propionate ratio, which elevates milk fat [3]. In confined cows, milk production usually increases with fat supplementation, although there is great variability in the level of response [8]. Finally, another variable to consider is genetics, since cows can be selected for high or low milk fat percentage [9]. More details about the types of fatty acids in milk fat after oil supplementation can be found in Johnson et al. [5].

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Milk protein is also sensitive to diet, but less than fat [1]. Increasing dietary protein increases total milk yield and total protein yield, but not the proportion of milk protein [4,10]. Feeding whole seeds and free oil may be effective in depressing the protein content in milk, but various studies report no effect [3,11].

Other variables to consider include the amount of feed consumed and the proportion of the nutrients absorbed into the bloodstream. Adding canola oil or essential oil decreases feed intake and digestibility [12], while conversely fish oil supplementation improves fiber digestibility [13].

Previous studies have found no effect of spent tea leaf [14], green tea waste [15] or decaffeinated tea waste [16] on milk yield or composition. There are no studies, however, on the effect of unspent tea leaf supplementation on milk composition. The present study intends to fill this gap by using tea leaf supplementation and comparing its effect to that of soybean oil supplementation.

The objective of the present research is to evaluate and compare the effect of soybean oil (SBO) and tea leaf (*Camellia sinensis*, TL) supplementation on milk yield and composition. These two supplementations were chosen because these two ingredients can aid in controlling CH_4 and Carbon dioxide (CO_2) emissions, which contribute to global warming [17]. Since these are not the only variables to consider when choosing a diet, it is also important to measure the effect of these supplementations on milk production and composition.

II. MATERIALS AND METHODS

The experiment was carried out at the Innovation and Practical Training Center, National Pingtung University of Science and Technology. The protocol for the research was approved by the University and done according to the established guidelines.

2.1 Animals

Three cannulated Holstein dairy cows on their first lactation were measured for feed intake, milk yield and composition. Every cow was considered an experimental unit. Each treatment was replicated 3 times, for a total duration of 21 days. The cows were separated from the herd and placed in individual pens (2 × 1.80 m).

2.2 Treatments

The three cows were fed with total mixed ration (TMR) (Table 1, Table 2) and had access to fresh water at all times. The amount of feed offered to the cows was 40 kg / day, divided in 3 meals at 06:30 (15 kg), 12:30 (10 kg) and 18:30 h (15 kg).

Table 1: Composition of TMR fed to milking cows

Ingredients	Proportion (%)*
Napier grass (*Pennisetum purpureum*)	42.75
Concentrate	14.23
Silage	42.75
$NaHCO_3$	0.35

*19% DM basis

Table 2: Concentrate composition included in the TMR

Ingredient	(%)
Corn meal	65
Soybean meal	15
Cracked corn	5
Wheat brand	10
Tallow	0.5
Ca	2.0
NaCl	1.0
NaHCO3	1.0
Premix	0.5
Chemical Composition	
DM	89%
CP	16.25%
ME	3 Mcal / kg
NEL	1.95 Mcal / kg
NDF	15.80%
NFC	60%
Fat	3.75%
UIP	35%

DM: Dry matter, CP: Crude protein, ME: Metabolizable energy, NEL: Net energy of lactation, NDF: Neutral detergent fiber, NCF: Non-fiber carbohydrate, UIP: Undegradable intake protein.

Supplementations of TL and SBO were tested and compared to the control treatment. The amount of supplement included in every treatment was determined after a pre-trial. Food was provided to a cow with different amounts of the supplements every day. Since adding the supplements affected feed palatability, the ideal dose of supplementation should be the maximum amount that does not severely compromise the cow's feed intake.

For the TL supplementation, 500 g of dried green tea leaves were provided in addition to the control diet, divided between the three meals (187.5, 125 and 187.5 g for the morning, noon and evening meal respectively). The green tea leaves were mixed evenly with the TMR before they were given to the cows. SBO supplementation consisted of 500 ml of soybean oil provided daily in addition to the control diet. This amount of soybean oil was divided between the three meals (187.5 ml, 125 ml, and 187.5 ml for the morning, noon and evening meal respectively). The soybean oil was poured on the top of the TMR.

2.3 Feed intake

Feed intake was measured every day. For all treatments, the feed residue was weighed 24 h after the first feeding so that the feed intake of the animals could be calculated.

2.4 Milking, sampling and equipment

Cows were milked twice a day (06:00 h and 18:00 h). The milking parlor had a herringbone design and the milking equipment was from Afimilk®. At each milking time, milk yield was automatically recorded from the milking parlor using AfiFarm Herd Management Software. Two milk samples were taken every day at each milking time for every cow. A total of 126 samples were taken for analysis. Potassium dichromate ($K_2Cr_2O_7$) was added to each sample (1 g $K_2Cr_2O_7$ / 100 ml milk) and kept at 4 °C for preservation until they were sent for fat, protein, lactose and solid non fat (SNF) analysis.

2.5 Volatile Fatty Acid (VFA) analysis

Ruminal liquor was collected from the cows 2 h after feeding (i.e., 08:30, 14:30 and 20:30 h). Immediately after collection, the ruminal liquor was strained through 2 layers of gauze and a solution of H_2SO_4 at 25% was added in order to drop the pH to 2. It was then stored at -20 ºC until VFA analyses were about to be done. Six hours before testing for VFA, the samples were thawed and put in small essay tubes (10 cc). The samples were centrifuged for 10 minutes at 3000 rpm in order to obtain a clear supernatant. After centrifugation, 5 cc of each sample was collected and put in a clean small essay tube.

The supernatant was analyzed for VFA concentrations. VFA [acetate (C2), propionate (C3), butyrate (C4), valerate (C5), isobutyrate (iC4), or isovalerate (iC5)] content of rumen fluid samples was measured via gas chromatography (GC) by using a GC machine (i.e., Hewlett Packard 5890).

Individual rumen VFA was separated using a fused silica capillary column (30 m x 0.53 mm ID, 1µm film thickness). An internal standard of 1 mL 30 mM 4-methylvaleric was prepared prior to the preservation of the sample. A split injection (50: 1) of 1 µl of ruminal liquor sample was injected into the GC machine. The injector and detector temperatures were 250 ºC. The initial oven temperature (125 ºC) was held for 5 min. It was then increased to 180 ºC at a rate of 15 ºC / min and held for 6 min. The total run time was 14 min per sample. High purity methanol was used as a carrier gas with a flow rate of 4.2 mL / min; input pressure was held constant. A Chem Data Station was used for the integration and quantification of the VFAs tested. A total of 189 samples were analyzed for VFA.

2.6 Experimental design and statistical analysis

A 3 × 3 Latin square design was used in this experiment. The data from the experiment were subjected to a general lineal model (GLM) and analysis of variance (ANOVA). The means were later compared for significance using Duncan's test at $P < 0.05$ [18].

III. RESULTS

Table 3 summarizes the results of feed intake, milk yield and composition for each treatment. Feed intake was not negatively affected by the addition of the supplements. Milk yield was significantly higher in the TL supplementation compared to SBO and the control treatment ($P < 0.05$).

Table 3. Feed intake, milk yield and milk quality per treatment.

Variable	N	Mean		
		Control	TL	SBO
Feed intake (kg)	21	23.25 ± 1.13^{a}	24.22 ± 1.71^{a}	23.49 ± 0.98^{a}
Milk yield (kg)	21	6.01 ± 0.44^{b}	6.65 ± 0.29^{a}	6.41 ± 0.35^{ab}
Fat (%)	84	2.81 ± 0.10^{b}	2.87 ± 0.12^{b}	3.19 ± 0.13^{a}
Protein (%)	84	3.49 ± 0.07^{a}	3.23 ± 0.06^{b}	3.56 ± 0.07^{a}
Lactose (%)	84	4.00 ± 0.07^{a}	3.72 ± 0.06^{b}	3.92 ± 0.07^{a}
SNF (%)	84	8.19 ± 0.14^{a}	7.65 ± 0.12^{b}	8.17 ± 0.14^{a}

Means with the same letter are not significantly different.
Means followed by different letters are significantly different at Duncan's multiple range test ($P < 0.05$).

Results show that both supplementations produced changes in milk composition. The percentage of fat in the milk increased significantly with SBO supplementation compared to the control and TL (See Table 3). TL supplementation negatively affected protein, lactose and SNF percentages ($P < 0.05$). Nonetheless, since milk yield was significantly higher for TL, one must consider dilution of other components in the percentage of water content when interpreting the results of the dietary change. As can be seen in Table 4, fat yield was significantly higher in SBO supplementation ($P < 0.05$). Protein, lactose and SNF yields did not show significant difference among treatments ($P > 0.05$) (See Table 4). Feed intake, milk yield or its composition did not change significantly according to day of treatment (See Table 5). This suggests that the effect of the supplements tested in this experiment was constant, and that the effect was effective since the first day of supplementation.

Individual and total VFA are shown in Table 6. C2 production was lower after SBO supplementation, compared to TL and the control treatment ($P < 0.05$). C3 was significantly higher after TL and SBO supplementation than in the control treatment ($P < 0.05$). C4 had the highest production after TL supplementation (1052.6 ppm) and lowest in the control treatment ($P < 0.05$). No significant differences were found in C5, iC4, or iC5 production among all treatments tested in this study ($P > 0.05$). As for ratios, SBO supplementation showed the lowest C2:C3+C4 and C2:C3, followed by TL supplementation and the control treatment ($P < 0.05$).

Table 4. Component yield and milk quality per treatment.

Component (g)	N	Mean		
		Control	TL	SBO
Fat yield	84	082.25 ± 3.84^{b}	90.35 ± 3.23^{ab}	99.08 ± 4.45^{a}
Protein yield	84	106.71 ± 4.82^{a}	108.95 ± 3.97^{a}	117.08 ± 4.93^{a}
Lactose yield	84	123.92 ± 51.66^{a}	125.21 ± 4.27^{a}	128.88 ± 5.32^{a}
SNF yield	84	251.66 ± 11.11^{a}	257.44 ± 8.62^{a}	268.37 ± 10.81^{a}

Means with the same letter are not significantly different.
Means followed by different letters are significantly different at Duncan's multiple range test ($P < 0.05$).

Table 5. Feed intake, milk yield and milk quality characteristics per day

Variable	N	Day						
		1	2	3	4	5	6	7
Feed intake (kg)	21	22.44 ± 2.68	22.53 ± 1.40	23.20 ± 1.88	22.89 ± 2.25	22.14 ± 1.91	25.65 ± 1.21	26.71 ± 1.17
Milk yield (kg)	9	6.68 ± 0.63	6.38 ± 0.48	6.55 ± 0.54	6.40 ± 0.56	6.19 ± 0.55	5.99 ± 0.38	6.32 ± 0.46
% Fat	36	2.95 ± 0.20	3.12 ± 0.15	2.75 ± 0.17	3.24 ± 0.22	2.82 ± 0.15	2.96 ± 0.20	2.86 ± 0.18
% Protein	36	3.30 ± 0.10	3.34 ± 0.11	3.37 ± 0.12	3.47 ± 0.11	3.45 ± 0.09	3.54 ± 0.11	3.52 ± 0.11
% Lactose	36	3.90 ± 0.10	3.82 ± 0.11	3.78 ± 0.12	4.00 ± 0.10	3.85 ± 0.10	3.92 ± 0.12	3.88 ± 0.12
% SNF	36	7.90 ± 0.19	7.86 ± 0.20	7.84 ± 0.23	8.17 ± 0.20	8.01 ± 0.19	8.16 ± 0.21	8.11 ± 0.22

All means are not significantly different at Duncan's multiple range test ($P < 0.05$).

Table 6. Volatile fatty acid production per treatment

VFA (ppm)	Control	TL	SBO
C2	5904.2 ± 106.95^{a}	6017.3 ± 131.08^{a}	5524.3 ± 123.46^{b}
C3	1295.64 ± 25.28^{b}	1581.56 ± 41.30^{a}	1629.68 ± 52.13^{a}
C4	878.28 ± 25.18^{b}	1052.6 ± 42.72^{a}	985.59 ± 41.09^{ab}
C5	212.44 ± 11.26^{a}	217.41 ± 12.41^{a}	216.85 ± 12.72^{a}
iC4	141.5 ± 7.71^{a}	160.4 ± 10.37^{a}	154.9 ± 10.19^{a}
iC5	201 ± 10.52^{a}	191.9 ± 11.05^{a}	200.79 ± 11.68^{a}
C2:C3+C4	2.73 ± 0.03^{a}	2.32 ± 0.05^{b}	2.18 ± 0.05^{c}
C2:C3	4.61 ± 0.07^{a}	3.90 ± 0.11^{b}	3.51 ± 0.09^{c}

Means with the same letter are not significantly different.
Means followed by different letters are significantly different at Duncan's multiple range test ($P < 0.05$).
C2: Acetate, C3: Propionate, C4: Butyrate, C5: Valerate, iC4: Isobutyrate, iC5: Isovalerate.

IV. DISCUSSION

In our study, fat percentage changed via nutrition, as Freeden [3] predicted. Nonetheless, he also predicts that polyunsaturated fatty acids lower fat percentage in milk. In our case, fat percentage is higher after SBO supplementation. The reason for the difference may be the warm, tropical climate in Southern Taiwan. Most studies regarding fat supplementation have been done in temperate climates, but Wang et al. [19] did a study in subtropical Taichung, Taiwan, which is located slightly above the Tropic of Cancer. They found that supplementation with both lard and prilled fat increased milk fat percentage in Holstein cows from 3.28 to 3.45 and 3.55% respectively, without affecting protein, lactose or SNF contents in milk. They suggest that heat stress played a key role in these results. Similarly, Kargar et al. [7] did a study in subtropical Iran. They found that, without affecting milk yield, both hydrogenated palm oil and yellow grease increased milk fat content from 3.62 to 3.77 and 3.86 respectively. Lin et al. [20] explain that Holstein cow milk yield decreases at an environmental

temperature between 21 and 27 °C and decreases significantly at a temperature above 27 °C. In our experiment, the average environmental temperature was 28.1 °C, the daily average high temperature was 31.9 °C and the mean relative humidity was 78%. Therefore, it can be said that the cows were under heat stress.

Another important difference is that studies in temperate climates show higher milk fat percentage those found in Taiwan. For example, Freeden [3] reports milk fat percentages ranging from 3.14 to 3.89 ± 0.18% in 28 herds from New Brunswick and 3.67 ± 0.004% in Quebec. These are considerably higher than those found by Wang et al. [19] in Taiwan and even more so for our study, where milk fat percentage was depressed in the control treatment due to heat stress. SBO supplementation was able to increase milk fat percentage almost to normal levels.

In our experiment, the C2 : C3 ratio was lower after SBO supplementation. According to Freeden [3], this is due to its antimicrobial effect. Wang et al. [19], the other study done in Taiwan, showed reductions of C2: C3 ratios from 3.74 to 3.51 and 3.56 respectively for lard and prilled fat. Kargar et al. [7] in Iran, on the other hand, found no change in C2 : C3 ratio after yellow grease and an increase from 2.6 to 2.9 after palm oil supplementation. An explanation for this is suggested by Freeden [3], who purports that if the fat supplement replaces dietary starch, the ruminal pH and the C2 : C3 ratio are both elevated. In Kargar et al [7], the pH was elevated from 6.02 to 6.24 and 6.21 after palm oil and yellow grease supplementation. This suggests that a higher ruminal pH hindered the antimicrobial effect of the fat supplement in both their supplementations and therefore kept the C2 : C3 from going down. Wang et al. [19], on the other hand, report ruminal pH levels of 6.69 and 6.60 respectively for lard and prilled fat, compared to 6.67 in the control treatment. Under heat stress, an extra boost of energy seems to have a greater effect over milk fat production than the VFA profile. The explanation given by Wang et al. [19] is that supplementation increases energy for lactation via 1) changing energy utilization pathways, 2) lower glucose consumption and 3) milk fat synthesis directly from fatty acids and glycerol in mammary glands. An increase of efficiency in the use of energy may also be improving lactation performance. According to a review of 11 studies and 15 trials [21], the efficiency of metabolizable energy for lactation increased by 2.7% when dietary lipid content increased from 3 to 6.9% of dry matter intake.

Schoeder et al. [8] purport that a positive energy balance is necessary to achieve maximum milk production response to fat supplementation, which helps explain why there is a lower increase in milk yield during early and mid-lactation. In our experiment, it seems that heat stress is severely hindering milk fat production. SBO supplementation would therefore be helping the cows balance their energy levels and helping them normalize the suppressed milk fat production.

We found a non-significant increase in protein content and yield after SBO supplementation. Reviews of previous research suggest that fatty acid (e.g., tallow, saturated fat, oilseeds and SBO) supplementation may depress protein content, but that other studies have reported no effect or even increases [3,11,21]. Conversely, Schingoethe [10] believes that feeding supplemental fat "invariably reduces milk protein content" with "all sources of supplemental fat feed". The explanation given is that increased mammary blood flow due to high fat supplementation prevents an increase in uptake of critical amino acids that are needed for improved milk synthesis efficiency [10,22]. The main concern here is that a milk protein reduction will affect farm revenue, given the recent emphasis of milk protein in pricing systems [8].

The purpose of spent tea leaf supplementation is that it can be a source of protein at the 8% level [14] or even at the 20% level [16]. Jarasuriya et al. [14] reported a non-significant increase in milk yield from 6.69 to 6.95 kg, while milk composition was similar to that in the control treatment. Decaffeinated tea waste is also high in polyphenols, which have little effect on milk yield and composition [16]. Therefore, we suggest that, in our experiment, the positive effect of tea leaf supplementation in milk yield was due to the caffeine content in the tea. This is plausible, since increased milk yield after caffeine supplementation has been observed in sows and rats [23]. Li and Hacker [23] suggest that this is due to "a highly functional mammary gland", which may be explained by 1) increased epithelial cell number and 2) increased cell activity. Further studies in cows may be needed to corroborate this.

In the present study, TL and SBO was supplemented because they have the potential to diminish CH_4 and CO_2 concentrations [17]. It is also important to mention that the study was done with low-yield cows during their first lactation. However, it is important to do research on medium and high-yield cows and on multiparous cows to see if these supplements have the potential to reduce GHGs and at the same time maintain or improve milk quality. It is also important to consider economic factors, since in some countries milk price is based on fat percentage (e.g., Taiwan), whereas in other countries buyers will pay more for more protein or less saturated fat in milk (e.g., in Europe).

In a review by Seymour et al. [24] using data from 20 studies, milk yield was found to be strongly related to rumen concentration of C4 and moderately related to C3 concentration, but not to rumen C2 or total VFA concentration. Therefore, in our experiment, the increase in milk yield after TL supplementation may be due to the increase in C4 and C3. On the other hand, SBO supplementation only produced a non-significant

increase in milk yield. The non-significant increase in butyrate and the increase in propionate were not enough to produce an increase in milk yield.

V. CONCLUSION

In conclusion, SBO supplementation in a warm, tropical climate increased milk fat percentage in Holstein cows without affecting milk yield or protein, lactose or SNF contents in milk. The C2 : C3 ratio was lower after SBO supplementation since, under heat stress, an extra boost of energy may have a greater effect over milk fat production than the VFA profile. On the other hand, a positive effect of tea leaf supplementation on milk yield was found, due to the caffeine content in the tea and an increase in rumen C4 and C3. These results suggest that SBO supplementation is a good alternative for solving a negative energy balance in Holstein cows due to heat stress in tropical countries, while also decreasing GHG emissions.

Acknowledgements

The work was supported by the Taiwan-ICDF scholarship and the Innovation and Practical Training Center at National Pingtung University of Science and Technology.

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