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Research Article
Effect of the Addition of Ca-PUFA Complexes to Complete Rations on Fermentability and Digestibility

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Abstract

Background and Objective: The purpose of this study was to assess the sources of polyunsaturated fatty acids (PUFAs) in supplemental feed for dairy cows. Samples obtained from three types of vegetable oils (soybean oil, corn oil and peanut oil) were selected to provide essential fatty acids to dairy cows. **Materials and Methods:** The approach of this study was to compare three types of vegetable oils added to rations to make Ca-PUFA complexes given to dairy cows. The parameters measured were fermentability and digestibility. The completely randomized design was used with six treatments and four replications. The treatments were: R1 = Ration +3% Corn Oil, R2 = Basal Ration + 3% Peanut Oil, R3 = Basal Ration +3% Soybean Oil, R4 = Basal Ration +3% Ca-Corn Oil Complex, R5 = Basal Ration +3% Ca-Peanut Oil Complex and R6 = Basal Ration +3% Ca-Soybean Oil Complex. **Results:** This study indicated that the production of NH_3 for the treatments ranged from 14.64±0.81 to 18.24±1.52 mM. The average volatile fatty acid (VFA) production ranged from 82.15±0.13 to 144.59±0.49 mM. The analysis of variance showed that the supplementation of Ca-oil complexes in complete rations increased VFA production values ($p<0.05$). Fatty acid compounding by calcium minerals improved digestibility compared to whole oil ($p<0.05$). Soybean oil compounded by calcium showed the highest fermentability and digestibility values among the three types of oils tested (corn oil, peanut oil and soybean oil). However, the results showed that peanut oil was comparable to soybean oil when considering economic factors. **Conclusion:** The use of peanut oil in the manufacturing of Ca-oil complexes can compete with soybean oil in terms of effectiveness.

Key words: Ca-PUFA, dairy cows, digestibility, fermentability, vegetable oils

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

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

INTRODUCTION

Fat is often included in rations given to dairy cows because it has a high energy density and low heat increment[1]. Fat is also added to rations to increase the absorption of fat-soluble nutrients, reduce the dusty character of the feed, increase the production and content of milk fat, as well as change the profile of milk fatty acids[2].

Fat content in forage feed is generally approximately 2-7% of dry matter, half of which is in the form of fatty acids[3]. Fat, as a non-polar compound, is not readily soluble in rumen fluid and tends to associate with feed particles and rumen microbes. This association allows the formation of a physical surface closure of fat[4]. Therefore, the addition of fat into rations can affect the rumen fermentation system.

Under conditions of fat blanketing, protozoan lipolytic activity is not as efficient as bacterial lipolytic activity. In this situation, protozoans are only able to hydrolyse phospholipids. Consequently, protozoan metabolic activity becomes disturbed in rumens with greater fat availability and this can cause protozoan mortality[5]. Livestock response to partial defaunation was shown by Sutardi *et al.*[6] to increase the growth of cows from 479-1,271 g day^{-1}.

The decline in the rumen protozoan population due to the addition of oil to rations varies based on the source of the fat. Linseed oil is very powerful in reducing the protozoan population[7]. Soybean oil (high in unsaturated fatty acids) is able to decrease the protozoa population in sufficient quantities[8] and high saturated fatty acid tallow sometimes has no effect[5]. Linolenic acid may be toxic to protozoa[3].

Some rumen bacteria have strong lipolysis ability against ration fat. This ability has been demonstrated by the presence of fat hydrolysis products in rumen without fatty acids, glycerol and galactose[9]. However, the growth of certain rumen bacteria, especially cellulolytic bacteria, decreases in the presence of ration fats while amylolytic species are less affected. However, the addition of fat does not alter the total concentration of bacteria in the rumen[3].

Calcium can reduce the negative effects of fat on carbohydrate digestibility[10]. Calcium binds to fatty acids to form insoluble calcium salts. The formation of calcium salts in the rumen were shown in *in vitro* experiments[11]. Fat supplementation can decrease the concentration of calcium ions in rumen fluid. Low levels of calcium become the limiting factor for bacteria to break down feed particles[12] and contributes to the decrease in digestibility of plant cell walls. There are indications that the polyunsaturated fatty acids (PUFAs) in calcium salts are in the hydrogenated form at low pH, while at pH 6-7, they are relatively stable. Van Nevel and Demeyer[8] reinforced the role of pH through *In vitro* techniques, that at pH above 6, soybean oil in the form of calcium salt-soybean oil are slightly hydrogenated. Unsaturated fatty acid hydrogenation in the rumen Ca-long chain fatty acid decreased by 30-40%[13].

Other vegetable oils, such as corn oil and peanut oil, can be used to make complexes with calcium minerals to form Ca-PUFA. These do not interfere with the rumen ecosystem, nor they are easily decomposed in post-rumen processes. The comparison of Ca-PUFA formation in some vegetable oils can be a novel reference for the use of vegetable oils in dairy cow rations.

Therefore, this study was carried out to obtain information on Ca-oil complex supplementation in animal feeds to determine beneficial mixtures which could for increasing dairy cow productivity.

MATERIALS AND METHODS

Materials and research tools: Vegetable oils, including soybean oil, peanut oil, or corn oil, were also part of the feed composition. The oils were by-products (waste products) of food material processing industries, such as tofu, *oncom* and processed corn. Research tools used included mixers, drying cabinets, scales and pans. Feed composition and nutrient content concentrations is presented in Table 1.

Research procedure: Peanut oil, corn oil and soybean oil were processed to get their respective saponification values. The amount of $Ca(OH)_2$ used was half the saponification number or 95.1 mg $Ca(OH)_2$ for each 1 g of oil. $Ca(OH)_2$ was dissolved in aquadest and stirred while being heated until homogeneous (20 min). The resulting soap was then mixed with dried dregs in a 1:1 ratio. When the mixture was dry the Ca-oil complex soap was ready to use.

Experimental design: The complete randomized design was used with six treatments and four replications. The treatments were:

R1 = Basal ration +3% Corn Oil
R2 = Basal ration +3% Peanut oil
R3 = Basal ration +3% Soybean oil
R4 = Basal ration +3% Ca-Corn Oil Complex
R5 = Basal ration +3% Ca-Peanut Oil Complex
R6 = Basal ration +3% Ca-Soybean Oil Complex

Differences in the results were resolved with Duncan's test.

Parameters measured:

- Supplemental fermentability measured by total NH_3 and VFA production
- Digestibility of dry matter and organic matter[14]

Statistical analysis: The results were recorded using Microsoft Excel 2016 software and statistical data was processed using SPSS 17 software. The analysis results are presented in Table 2 for comparison of the significant differences.

RESULTS AND DISCUSSION

The measurements of fermentability and digestibility of rations with whole oil and oil mineral complex supplements are presented in Table 2.

Based on Table 2, the production of NH_3 for the various treatments ranged from 14.64±0.81 to 18.24±1.52 mM. The NH_3 values tended to be high in each treatment but was still tolerable by the cattle. The optimum rumen NH_3 concentration range is 4-12 mM[15].

The analysis showed that the soybean oil additions either as a whole oil (P3) or made into a complex with calcium minerals (P6) produced NH_3 at a higher concentration than other treatments. Both P3 and P6 yielded similar NH_3 concentrations. The lowest NH_3 concentrations were obtained when using corn oil additions (P1 and P4). However, NH_3 production in treatment P4 was higher than that in treatment P1 ($p<0.05$). This finding indicates that the corn oil with the addition of the calcium complex was effective in maintaining the rumen microbial population. Similarly, peanut oil with the addition of the calcium complex also had an impact on improving the rumen ecosystem.

Ca-oil complex additions were significantly different based on the analysis of variance. The NH_3 content ranged from 14.64±0.81 to 18.24±1.52 mM. These high NH_3 values can still support the growth of rumen microbes.

Volatile fatty acid production is the result of anaerobic fermentation of organic matter ration, especially carbohydrates in the rumen. Under normal circumstances, the total VFA concentration in rumen fluid ranges from 70-150 mM according to Sutardi[15]. Total VFA concentration in rumen fluids for optimal microbial growth is 80-160 mM. The average VFA production of the treatments varied: P1 (82.15±0.13), P2 (116.36±0.43) P3 (123.65±0.32), P4 (121.67±0.64), P5 (138.03±0.33) and P6 (144.59±0.49) mM; thus, the values ranged from 82.15±0.13 to 144.59±0.49 mM. The analysis of variance indicated that Ca-oil complex supplementation in complete rations influenced the VFA value ($p<0.05$). The lowest VFA production occurred during the P1 treatment, while P5 and P6 treatments had the highest production of VFAs. When comparing VFA production between supplementation with whole oil (P1, P2, P3) and oil complexes with calcium (P4, P5, P6), the former treatments result in lower VFA production than the latter ($p<0.05$). This finding indicates that oil-mineral complex manufacturing is effective in improving the rumen ecosystem because oil complexes with calcium do not disturb the rumen ecosystem. Oil-mineral complex addition generally results in higher total VFA production than whole oil additions.

VFA content in rumen fluid is a measure of feed fermentability and is closely related to rumen microbial activity. The required VFA_{Total} level to support optimal cattle growth is 80-160 mM[15]. During this study, VFA values in each treatment were in the normal range to support optimal cattle growth.

Table 1: Feed composition and nutrient content concentrations

No.	Feed ingredients	Percentage
1	Wheat pollard	22.00
2	Soybean meal	25.00
3	Cornmeal	50.00
4	Molasses	2.00
5	Fishmeal	1.00
Total		100.00
Nutrient contents		
1	Dry matter	88.70
2	Crude protein	18.40
3	Crude fat	5.85
4	Crude fibre	9.48
5	TDN	75.00
6	Calcium	0.23
7	Phosphate	0.63

Table 2: Fermentability and digestibility of dry and organic material rations

	Treatments					
Variables	P1	P2	P3	P4	P5	P6
NH_3 (mM)	14.64±0.81^{d}	16.02±9.66^{bc}	18.24±1.52^{a}	15.71±3.79^{c}	16.55±3.74^{b}	17.75±3.16^{a}
VFA_{total} (mM)	82.15±0.13^{c}	116.36±0.43^{b}	123.65±0.32^{b}	121.67±0.64^{b}	138.03±0.33^{a}	144.59±0.49^{a}
DMD (%)	54.42±0.54^{f}	58.90±0.92^{e}	63.10±0.92^{c}	60.42±0.25^{d}	64.41±0.25^{b}	65.94±0.56^{a}
OMD (%)	55.33±0.46^{e}	60.03±1.13^{d}	64.24±1.74^{c}	60.98±0.63^{d}	66.05±0.57^{b}	68.11±1.36a

Different superscripts in the mean columns indicates statistically significant differences ($p<0.05$). P1: Basal Ration +3% Corn oil; P2: Basal ration +3% Peanut oil, P3: Basal ration +3% Soybean oil, P4: Basal ration +3% Ca-corn oil complex, P5: Basal ration +3% Peanut Ca-peanut oil complex, P6: Basal ration +3% Ca-soybean oil complex

The average dry matter digestibility (DMD) values were P1 (54.42±0.54%), P2 (58.90±0.92%), P3 (63.10±0.92%), P4(60.42±0.25%), P5 (64.41±0.25%) and P6 (65.94±0.56%). In general, the values of DMD in the mineral oil complex addition treatments (P4, P5 and P6) were higher than the whole oil addition treatments (P1, P2 and P3). According to Tillman *et al.*[16] the factors affecting digestibility include feed substance composition, crude protein digestibility, fat, food preparation, amount of food, animal factors and ration composition. The Ca-oil complex addition showed higher DMD values than the addition of oil without Ca saponification.

High DMD values are an indication of a balanced rumen ecosystem. A reduction in rumen microbes reduces digestibility. The supplementation of the rations with Ca-oil complexes did not disturb the rumen ecosystem, thus DMD values were greater than 60%.

Organic matter digestibility (OMD) is closely related to DMD since some dry matter was organic matter composed of crude protein, coarse fat, crude fibre and Nitrogen-Free Extract. The results showing the effect of Ca-oil complex additions on Organic Matter Digestibility (OMD) are presented in Table 2.

The average OMD values were P1 (55.33±0.46%), P2 (60.03±1.13%), P3 (64.24±1.74%), P4 (60.98±0.63%), P5 (66.05±0.57%) and P6 (68.11±1.36%). The average OMD value in each treatment ranged from 55.33±0.46 to 68.11±1.36%. The OMD values of whole oil rations (P1, P2, P3) were generally lower ($P<0.05$) than oil complexes with calcium (P4, P5, P6). These results indicated that the process of compounding fatty acids with calcium minerals could improve OMD compared to OMD with whole oil. Among the three types of oils (corn oil, peanut oil and soybean oil), soybean oil with the calcium complex produced the highest OMD values.

The OMD and DMD levels were similar. Increases in DMD are often in line with increases in OMD since most of the dry matter in rations is composed of organic matter, so factors influencing DMD would also influence OMD[17]. Overall, the DMD and OMD values in Ca-oil complex supplements were greater than values in treatments without calcium complexes.

The implication of this study is that Ca-oil complex supplementation is better than whole oil supplementation in dairy cow rations. Ca-soybean oil shows the best dairy cow fermentability and digestibility but soybean oil is quite expensive, so the use of Ca-peanut oil complex is recommended. Overall, complex Ca-peanut oil supplementation can be applied to dairy farms.

CONCLUSION

Supplementation with Ca-oil complexes is better than supplementation with whole oils in terms of fermentability and digestibility of the rations given to dairy cows. The use of peanut oil for manufacturing Ca-oil complexes can compete with soybean oil in terms of cattle growth and cost effectiveness.

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