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Effects of Different Levels of Fish Oil and Canola Oil on *in vitro* Dry Matter and Organic Matter Digestibility

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Abstract: This experiment was designated to examine the effects of different levels of fish oil and canola oil on *in vitro* dry matter and organic matter digestibility of two forages including alfalfa hay and corn silage. Using a completely randomized design with 3×3 factorial arrangement of treatments, the effects of three levels (2, 4 and 6% of DM) of three oil sources (Fish Oil (FO), Canola Oil (CO) and combination of Fish Oil and Canola Oil (FOCO) in 50:50 ratios) in an *in vitro* batch fermentation on Dry Matter (IVDMD) and Organic Matter (IVOMD) digestibility of alfalfa hay and corn silage were studied. In this experiment, in contrast with control, all oil sources decreased IVDMD (71.4, 69.1, 66.2 and 70 for control, FO, CO and FOCO, respectively) and IVOMD (69.98, 66.4, 63.2 and 68.4 for control, FO, CO and FOCO, respectively) of alfalfa hay significantly ($p<0.01$). For corn silage, IVDMD (64.3, 63.5, 59.1 and 63% for control, FO, CO and FOCO, respectively) and IVOMD (65.4, 61.9, 58.4 and 62.5% for control, FO, CO and FOCO, respectively) decreased significantly, when oil was added. Among oil sources, canola oil significantly ($p<0.05$) decreases IVDMD and IVOMD of both forages in comparison with two other oil sources. Increasing oil levels significantly ($p<0.05$) decreased IVDMD and IVOMD of both forages but alfalfa hay was more susceptible to increasing oil levels than corn silage.

Key words: Fish oil, canola oil, *in vitro*, digestibility, alfalfa, corn silage, Iran

INTRODUCTION

The energy requirement of the modern cow, which has been bred for enhanced milk yield is difficult to meet during early lactation. Due to the high energy value of fat, supplemental fat is frequently added to ruminant diets to increase their energy density (Oldick and Firkins, 2000; Juchem *et al*., 2008). Although, dietary fat increase energy density of diets, its influence on nutrient supply to the animal depend on the digestibility of the fat sources and effects of supplemented fat on intake, rumen fermentation and the other diet component digestibility (Khorasani and Kennelly, 1998). Ruminal fermentation and fiber digestion often decrease by addition of fats or oils to the diet; however degree of inhibition varies with amount and type of supplemented fats (Jenkins and Jenny, 1992). In comparison with saturated fatty acids, unsaturated fatty acids, which is more presented in plant oils than animal fat sources, had more inhibitory affects on ruminal ecosystem (Jenkins, 1993; Pantoja *et al*., 1994), decreased dry matter intake (Harvatine and Allen, 2006) and fiber digestibility especially in high concentrate diets (Ueda *et al*., 2003). Consequently, the proportion of unsaturated and the ratio of unsaturated fatty acids to saturated ones appear to exert the most influence on ruminal fermentation (Oldick and Firkins, 2000).

Various *in vivo* and *in vitro* methods have been used to measure the extend of nutrient digestion in ruminant animals. The *in vitro* Tilley and Terry (1963) procedure has been extensively used to measure Dry Matter Digestibility (IVDMD) and Organic Matter Digestibility (IVOMD) of feedstuffs and has been the most accurate and practical laboratory method available for prediction of digestibility data for ruminants (Mabjeesh *et al*., 2000). This method has been modified and adapted for starch feeds analysis and various researchers have improved its accuracy of prediction.

Objectives of these experiments were to determine the effects of different levels of fish oil and canola oil and their combination on *in vitro* digestion of alfalfa hay and corn silage as basal forages.

MATERIALS AND METHODS

Using a completely randomized design with 3×3 factorial arrangement of treatments, the effects of three

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levels (2, 4 and 6% of DM) of three oil sources (fish oil, canola oil and combination of fish oil and canola oil in 50:50 ratios) in an *in vitro* batch fermentation (Tilley and Terry, 1963) were studied. Treatments were assigned once to alfalfa hay and once to corn silage as forage basic, which were dried in a force-air oven at 60°C and milled to pass a 2 mm screen using a wiley mill (Arthur H. Thomas, Philadelphia, PA) and were analyzed for CP, ADF and NDF.

The alfalfa hay had the following chemical composition (DM basis): 16% CP, 52.5% NDF and 35.5% ADF. The corn silage contained (DM basis): 6.9% CP, 43.2% NDF and 21.5% ADF. For each forage source, three tubes were used as control without oil supplements. Kilika fish oil (khazar Co, Babolsar, Iran) and canola oil (Golestan Soybean Co, Gorgan, Iran) were used in this experiments. Using gas chromatography method, fatty acid composition of diets and supplemental oil has been determined, which are shown in Table 1.

Incubation was performed using rumen fluid from two rumen fistulated sheep maintained on a diet of hay. The liquor was taken before morning feeding, strained through two layers of cheesecloth into a flask and kept under CO_2 gas at 38-39°C until used. The incubation was conducted in 90 mL tube containing 1 g (W_1) of milled alfalfa hay or corn silage, which were previously supplemented with different levels of oils, 10 mL of rumen fluid and 40 mL of phosphate bicarbonate buffer (McDougall, 1948). Each tube was gassed with CO_2 before sealing with rubber corks with a gas release valve. Then tubes were incubated at 38°C for 48 h with occasional shaking.

At the end of the first incubation period, 6 mL HCL (20%) and then 5 mL pepsin solution (0.5 g pepsin was solved in 100 mL HCL 0.1 N) were added gradually to each tube.

The tubes were then incubated at 38°C for 48 h. Finally, the supernatant were discarded using filter studies (No. 42), the residues then were transferred to a 50 mL cruse and dried at 55°C until constant weight. The dry weight of the residue were calculated (W_2) to measure IVDMD and for IVOMD measurement, dried samples were heated at 550°C for 3-4 h. After that samples, weight was recorded (W_3). Using following formula, IVDMD and IVOMD were calculated.

$$\text{IVDMD} = \frac{W_1 - W_2}{W_1}$$

and

$$\text{IVOMD} = \frac{W_2 - W_3}{W_2}$$

Table 1: Fatty acid composition (g/100 g of fatty acids) of supplemental oils

Fatty acids	[1]Fish oil	[2]Canola oil
C12:0	0.29	0.22
C14:0	4.42	-
C16:0	24.04	4.20
C16:1	5.99	0.25
C18:0	5.11	2.26
C18:1 (n-9)	33.64	63.75
C18:2 (n-6)	3.20	16.92
C18:3 (n-3)	1.33	8.00
C20:0	0.22	0.69
C20:5 EPA	5.07	-
C22:6 DHA	13.32	-

[1]Khazar Co, Babolsar, Iran; [2]Golestan Soybean, Gorgan, Iran

RESULTS AND DISCUSSION

Results of IVDMD and IVOMD of oil supplemented alfalfa hay and corn silage are presented in Table 2 and 3, respectively. In contrast with control treatments of these two forages, including fish oil, canola oil and their combination decrease IVDMD and IVOMD of both forages significantly ($p<0.01$). IVDMD and IVOMD of alfalfa hay and corn silage decreased significantly in CO treatments in comparison with two other oil sources ($p<0.05$) and linear increase in oil levels from 2-6% (DM) cause IVDMD and IVOMD decrease in alfalfa hay but in corn silage including 6% oil in comparison with 2% decrease IVDMD and there were no significant differences in dry matter digestibility, when oil level rise from 2-4% and from 4-6%. IVOMD of corn silage tend to be decrease, when oil level increased from 2-6% ($p = 0.06$). Oil levels and sources interactions were not significant for both forage type ($p>0.05$). The *in vitro* method of evaluating the digestibility of ruminant feeds is used worldwide. The method is easier than *in vivo* analysis and avoids the need to surgically prepared animals in different locations in the gastrointestinal tract. The TT method has also been proven more accurate than digestibility predictions based on the chemical compositions of feeds (Van Soest and Wine, 1967). Moreover, because of its similarity to *in vivo* values (Tilley and Terry, 1963), it is considered a reference method for the prediction of ruminant feed digestibility. Fiber degradation in the rumen may be limited by several dietary factors that alter attachment by or nutrient availability to fibrolytic bacteria. Cellulolytic bacteria apparently need to be in close proximity or attached to fiber before degrading it. Among several theories discussed by Palmquist and Jenkins (1980), one states that unsaturated dietary fats may coat fiber and interfere with bacterial attachment. As revealed in results, unsaturated oil supplementation significantly declined IVDMD and IVOMD of both forage type, which is mostly related to NDF and ADF section of forages. Including unsaturated oil in diets often decrease

Table 2: The effects of different levels of fish oil and canola oil on *in vitro* digestibility of alfalfa hay

	Parameters[1]			
Treatments	IVDMD	SE	IVOMD	SE
Control	71.4	1.06	69.98	0.91
Oil[2]				
FO	69.1[a]	0.83	66.4[a]	0.81
CO	66.2[b]	-	63.2[b]	-
FOCO	70[a]	-	68.4[a]	-
p-value	0.01	-	0.001	-
Level (DM%)				
2	70.8[a]	0.77	68.7[a]	0.80
4	68.4[b]	-	66[b]	-
6	66[c]	-	63.4[c]	-
p-value	0.001	-	0.001	-
p-value				
Contrast				
Control vs. others	0.03	-	0.01	-

Table 3: The effects of different levels of fish oil and canola oil on *in vitro* digestibility of corn silage

	Parameters[1]			
Treatments	IVDMD	SE	IVOMD	SE
Control	64.3	0.79	65.4	1.4
Oil[2]	-	-	-	-
FO	63.5[a]	0.51	61.9[a]	0.62
CO	59.1[b]	-	58.4[b]	-
FOCO	63[a]	-	62.5[a]	-
p-value	<0.0001	-	0.001	-
Level (DM%)				
2%	62.9[a]	0.49	62.1[a]	-
4%	61.9[ab]	-	60.9[ab]	-
6%	60.7[b]	-	59.8[b]	-
p-value	0.04	-	0.06	-
p-value				
Contrast	-	-	-	-
Control vs others	0.01	-	0.003	-

[1]IVDMD = *In vitro* Dry Matter Digestibility, IVOMD = *In vitro* Organic Matter Digestibility; [2]: FO = Fish Oil, CO = Canola Oil, FOCO = Fish Oil + Canola Oil (50:50)

digestibility of nutrients, spatially in forages (Avila *et al*., 2000). IVDMD of soybean oil supplemented broomgrass hay decreased significantly and similar to our results, a linear decline was noted for IVDMD of this hay as the percentage of soybean oil in diet increased from 3-6% (DM) (Whitney *et al*., 2000). However, addition of 10% (DM) of soybean oil or hydrogenated tallow did not affect *in vitro* NDF digestibility of crystalline cellulose (Firkins *et al*., 1991), which has been related to the basal diet used. Including canola oil in comparison with fish oil or combination of fish oil and canola oil significantly decreased IVDMD and IVOMD of both forages, which was probably because of higher ratio of unsaturated fatty acids to saturated fatty acids (U:S) in canola oil in comparison with fish oil. As reported previously (Avila *et al*., 2000) oil suplements with higher U:S ratio will have more adverse effect on fiber digestibility. Alfalfa hay and corn silage had shown different responses of digestibility to oil levels. Increasing levels of oil from 2-4 and from 4-6 (DM%) significantly decreased IVDMD and IVOMD of alfalfa hay but IVDMD of corn silage was declined significantly when oil level increased from 2-6%. One of the possible reasons of these differences might be chemical composition of alfalfa hay and corn silage. Alfalfa hay had higher percentages of ADF and NDF than corn silage, which made it more sensitive to increasing oil levels. Results of this experiments show that unsaturated oils significantly decreased IVDMD and IVOMD of forages and their depends on oil type, oil levels, degree of saturation, basal diet and chemical compositions of forage.

CONCLUSION

This study shows that oil sources, oil levels, basal diets and degree of oil saturation are the most important factors affecting nutrient digestibility of forages. *In vitro* results revealed that in control with no supplemented diets, oil included treatments had less dry matter and organic matter digestibility *in vitro*.

Among oil sources, canola oil had more negative effects of alfalfa hay and corn silage and digestibility of alfalfa hay was more decreased than corn silage when oil levels were increased.

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