Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Fat and Fatty Acid Digestibility in Blue Foxes (*Alopex lagopus*) Fed Non-Supplemented and Inulin-Supplemented Diets

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ARTICLE HISTORY (14-108)

Received: February 22, 2014
Revised: January 05, 2015
Accepted: May 23, 2015

Key words:
Blue fox
Fatty acids
Inulin
Total digestibility

ABSTRACT

The research aimed to assess fat and fatty acid (FA) digestibility in foxes fed a standard diet without (0%) or with addition of low levels of inulin (0.25; 0.5 and 1% of diet, as-fed basis). Twenty-four blue foxes were divided into four treatment groups. The chemical composition of the diet and dietary and fecal FA profile were analyzed. The main dietary FAs were: palmitic (C16:0) and oleic (C18:1n-9) (38.63 and 32.50% total FAs). Linoleic acid (C18:2n-6) was the most abundant dietary polyunsaturated fatty acid (PUFA) (87%). Dietary fat and majority of individual FAs were almost completely digested (>97%) regardless of the diet used. The lowest digestibility was shown by long-chain saturated fatty acids (SFA) (C22:0 and C20:0). Addition of 0.5% inulin decreased ($P<0.05$) the digestibility of monounsaturated fatty acids (MUFA) compared to the control and 0.25% inulin diet. Supplementation of 0.5 and 1% inulin decreased ($P<0.05$) the digestibility of most abundant dietary SFA (C16:0 and C18:0), some MUFA (C16:1n-7, C20:1n-11) and the derivatives of essential FAs from the n-6 family (C20:3 and C20:4) but had no effect on the absorption of the parent forms of essential FAs (C18:2n-6 and C18:3n-3). The present study gives preliminary information on the effect of inulin on lipid digestibility in carnivorous animals so further investigations are needed to confirm our findings.

To Cite This Article: Burlikowska K, R Szymeczko, A Piotrowska and A Kułakowska, 2015. Fat and fatty acid digestibility in blue foxes (*Alopex lagopus*) fed non-supplemented and inulin-supplemented diets. Pak Vet J, 35(4): 451-455.

INTRODUCTION

The blue fox (*Alopex lagopus*) has a simple gastrointestinal tract, relatively short intestine and fast passage rate so in order to satisfy its living and reproductive needs and to develop high-quality fur, requires diets of a high concentration of energy and protein. Because of their favorable costs compared to other nutrients, dietary fats constitute the most efficient source of energy in feeds for fur animals. Depending on the feeding period, in a properly balanced diet for foxes, fat provides a considerable amount (up to 55%) of metabolizable energy (ME) (Enggaard Hansen, 1992). Moreover, dietary fat adds palatability and acceptable texture to feed, promotes the absorption of fat soluble vitamins and supply essential fatty acids (EFAs) that cannot be synthesized in the animal organism (Bauer, 2008; Case *et al*., 2011). Fatty acids (FAs) are a substantial part of lipids constituting 95% of a fat particle. Besides being the major fuel for the organism, they are also structural and functional components of cell membranes, precursors for lipid mediators, components affecting signal transduction pathways and gene transcription (Nguyen *et al*., 2009; Case *et al*., 2011). The main factors determining fat digestibility are: feed ingredients, type of dietary fat and different physicochemical properties of FAs such as carbon chain length, unsaturation number and FA position on glycerol (Doreau and Chilliard, 1997; Ramírez *et al*., 2001). Foxes are genetically adopted to utilize dietary fat efficiently, which is reflected in their high fat digestibility coefficients (generally over 90%) noted when ileal and total digestibility was measured (Burlikowska and Szymeczko, 2007; Gugołek *et al*., 2010).

One of many factors affecting the digestibility of fat in monogastric animals is the level and type of dietary fiber. Inulin is a kind of fermentable carbohydrates resistant to hydrolysis by mammalian enzymes. Studies

conducted so far show that inulin-type fructans, defined as prebiotics, positively affect intestinal microbiota, decrease fecal odor components, reduce blood lipids, enhance mineral absorption and positively stimulate the immune system (Delgado *et al*., 2010; Curbelo *et al*., 2012). In canine, the main factors conditioning the effects of fructans supplementation are their form and level at which they are incorporated into the diet. According to Propst *et al*. (2003), the ideal low-level supplementation rate for dietary fructans should ensure optimal nutritional benefits, few if any adverse side-effects on the host, and finally allow for the production of an economical diet that seems even more important in case of fur-bearing than companion animals. The influence of dietary fructans on the nutrient digestibility in dogs and cats was studied but the results obtained are inconsistent (Hesta *et al*., 2001; Propst *et al*., 2003; Barry *et al*., 2009).

In the available literature, there is no information regarding the influence of dietary fructans on nutrient digestibility in carnivorous fur animals. Therefore, the present research aimed to estimate fat and FA digestibility in blue foxes fed a standard diet without and with low-levels of inulin.

MATERIALS AND METHODS

All animal care procedures were approved by the Local Ethical Committee on Animal Testing at the University of Technology and Life Sciences in Bydgoszcz (No. 2/2010). The digestibility experiment was conducted in February 2010.

Animals and diets: Twenty-four 9-month-old blue fox males (mean BW 9.15±0.15 kg) were kept in a temperature-controlled room (12°C), housed individually in stainless steel cages (81 x 65.5 x 65.5 cm) with wire mesh floors and drip pans for excreta collection and had unlimited access to water. A 10h light: 14h dark cycle was applied.

The animals were fed a standard diet which was formulated to meet or slightly exceed the maintenance requirement of adult foxes (NRC, 1982). Dietary chemical composition, energy content and ME distribution from protein fat and carbohydrates are presented in Table 1. The standard diet was based on fish and poultry offal, meat meal and extruded cereals. Dried beet pulp was included as moderately fermentable fiber. The control diet had no added inulin. Three experimental diets were supplemented with increasing levels of inulin (FRUTAFIT® IQ ORAFTI, Belgium) 0.25, 0.5 and 1% (as-fed basis) added at the expense of beet pulp. Chromic oxide (Cr_2O_3, 5g kg^{-1}) was used as a digestion marker. The diets were homogenized, divided into daily portions (376.81 kJ ME kg^{-1} of BW; NRC, 1982) and stored at -25°C until the beginning of the feeding experiment. The animals were fed once daily (8:00 h).

Foxes were randomly assigned to one of four diets in order to create treatment groups with similar BW. Total duration of the digestibility experiment lasted for 16 days: adaptation (12 days) and total collection of feces (4 days). Feces excreted during the collection phase were removed from the trays once a day in the morning, weighted and stored in plastic boxes at -25°C for subsequent analyses. After the termination of the experiment, samples of the diets and feces were lyophilized and milled before further analyses.

Chemical analyses: The standard diet was analyzed for dry matter, crude protein (Kjeldahl-N x 6.25), ash and crude fiber according to AOAC (2010). The lipid content in the diet and feces was determined by the gravimetric method according to AOAC (2003) with a slight modification. A sample (approx. 0.5 g) was extracted with diethyl ether (4 ml) within 24 hours. The extraction procedure was repeated four times. The collected extracts were evaporated under nitrogen and dried at 105°C for 3 hours. Chromic oxide in the diet and feces was estimated by the method described by Kimura and Miller (1957).

Fatty acid composition of the diet and feces was analyzed after the extraction with chloroform-methanol (2:1) (Folch *et al*., 1957). The extracted lipids were hydrolyzed with 0.5M NaOH and esterified with 14% boron trifluoride methanol solution to obtain fatty acid methyl esters (FAMEs). FAMEs were analyzed by gas chromatography-mass spectrometry (GC-MS) using a PerkinElmer CLARUS 600 GC/MS model equipped with a PerkinElmer Elite-5MS capillary column (60m, 0.25 mm diameter, 0.5 µm film thickness). The temperature program was: from constant 140ºC for 4 min, with 4ºC/min gradient, to 270ºC (constant) for 5 min. The following parameters were applied: carrier gas He 6.0 (flow rate 1ml/min), split-less injector temperature 250ºC, transfer line temperature 290ºC, ion source temperature 250ºC. All the analyses were made in duplicate by the total ion current, TIC (MS scanning m/z: 35-400 Da).

Calculations: The content of N-free extractives was calculated by difference:

N-free extractives = dry matter – (crude protein + crude fat + crude fiber + crude ash)

Metabolizable energy from protein, fat and carbohydrates was calculated with the use of factors given by Enggaard Hansen (1992).

Apparent total fat and FA digestibility was calculated by the following equation:

$$\text{Digestibility (\%)} = 100\% - \left(\frac{\text{\% Chromic oxide in diet}}{\text{\% Chromic oxide in feces}} \times \frac{\text{\% Fat/Fatty acid in feces}}{\text{\% Fat /Fatty acid in diet}} \times 100\%\right)$$

Statistical analysis: Data were analyzed using the STATISTICA 8 software (StatSoft, Inc.®). The statistical comparison was made among four treatment groups. For the data that met the assumption of homogeneity of variance (Leven's test) and normal distribution (Shapiro-Wilk's W test) one-way ANOVA and the *post hoc* Tukey's test were used. The results are expressed as the mean and SEM. The nonparametric Kruskal-Wallis' test was used when variances were not homogeneous or when the data did not pass the normality test. The Kruskal-Wallis' test was followed by Dunn's nonparametric multiple comparison procedure. The data are presented as medians with range. Differences were considered significant at $P<0.05$.

Table 1: Ingredient and chemical composition of the standard blue fox diet

Item	Standard diet
Ingredient, g kg^{-1} as fed	
Fish offal	400
Poultry offal	223
Meat meal	60
Extruded cereals	100
Dried beet pulp	20
Vit.-min. Mix*	2
Water	195
ME, kJ kg^{-1}	5025
ME distribution (%) from:	
Protein	37
Fat	40
Carbohydrates	23
Chemical composition, g kg^{-1} DM	
DM, g kg^{-1} as fed	253
Crude protein	368
Crude fat	175.5
Crude fiber	22
Ash	123
N-free extractives	269.4

*Guyofox Plus, concentration per 1 g: vit. A 3000 IU; D_3 300 IU; E 50 mg; K 0.5 mg; B_1 22 mg; B_2 3 mg; B_6 3 mg; B_{12} 0.02 mg; H 0.03 mg; folic acid 0.3 mg; PP 5 mg; calcium panthotenate 3.15 mg; choline chloride 50 mg; Mn 7.5 mg; Zn 10 mg; organic Fe 20 mg; non-organic Fe 4.8 mg; Se 0.058 mg; Cu 1.25 mg; Co 0.01 mg.

Table 2: Fatty acid composition (% total fatty acids) of the standard blue fox diet

Fatty acid (common name)	Standard diet
C 14:0 (myristic)	2.60
C 15:0 (pentadecylic)	0.23
C 16:0 (palmitic)	38.63
C 17:0 (margaric)	0.20
C 18:0 (stearic)	5.66
C 20:0 (arachidic)	0.04
C 21:0 (heneicosylic)	0.01
C 22:0 (behenic)	0.01
C 24:0 (lignoceric)	0.01
SFA	47.63
C 14:1n-5 (miristoleic)	0.06
C 16:1n-7 (palmitoleic)	10.29
C 18:1 n-9 trans (elaidic)	4.90
C 18:1n-9 (oleic)	32.50
C 20:1n-11 (gondoleic)	0.45
C 22:1n-9 (erucic)	0.01
C 24:1n-9 (nervonic)	0.01
MUFA	48.22
C 18:2n-6 (linoleic)	3.62
C 20:3n-6 (dihomo-γ-linoleic)	0.46
C 20:4n-6 (arachidonic)	0.02
Total n-6	4.10
C 18:3n-3 (α-linolenic)	0.01
C 20:3n-3 (eicosatrienoic)	0.02
Total n-3	0.03
PUFA	4.15

RESULTS

Fatty acid composition of the dietary fat is presented in Table 2. Saturated (SFA) and monounsaturated fatty acids (MUFA) constituted more than 95% of total fatty acids (TFA). The main SFA were palmitic (C16:0), stearic (C18:0) and myristic acid (C14:0) (81, 12 and 5% SFA, respectively). The main MUFA were oleic (C18:1n-9), palmitoleic (C16:1n-7) and elaidic acid (C18:1n-9 trans) (67, 21 and 10% MUFA). The content of polyunsaturated fatty acids (PUFA) slightly exceeded 4% of TFA. Linoleic acid (C18:2n-6) was the main PUFA (87% PUFA).

During the whole experiment, the examined diets were well accepted by the animals and had 100% intake. The control blue foxes had very high (>99%) digestibility coefficients of fat and majority of determined FAs (Table 3). The lowest digestibility values in the inulin-free diet were noted for long-chain SFA i.e. C22:0 and C20:0. Among MUFA, nervonic acid (C24:1n-9) had the lowest digestibility. There were no significant differences among treatments in digestibility of fat, SFA and PUFA (Table 3). Supplementation of diets with 0.5% inulin significantly reduced MUFA digestibility compared to the control and 0.25% inulin diet. However, the highest amount of inulin (1%) had no effect on MUFA digestibility.

Digestibility coefficients of almost all individual FAs of the diets with inulin exceeded 97% except for C24:1n-9, C20:0 and especially C22:0, which showed the lowest digestibility coefficients (52.67-73.61%). Palmitic (C16:0) and stearic (C18:0) acids had significantly lower digestibility in the diets supplemented with 0.5 and 1% inulin compared to the control and 0.25% inulin groups. The negative effect of 0.5 and 1% inulin on FA digestibility was also noted in case of some MUFA (C16:1n-7 and C20:1n-11) and PUFA from n-6 family (C20:3 and C20:4). Digestibility of C21:0, C14:1n-5 and C22:1n-9 was significantly lower in 0.25 and 0.5% inulin diets but the highest level of inulin (1%) had no effect on their digestibility coefficients.

DISCUSSION

The main dietary ingredient commonly used as a source of ME for carnivorous fur-bearing animals is fat, mostly of animal origin, digestibility of which varies depending on different dietary factors and animal species. The digestibility coefficients of fat contained in standard feeds for foxes based on different animal offal generally exceed 95% (Burlikowska and Szymeczko, 2007; Gugołek *et al*., 2010). In the present experiment, blue foxes were fed diets based on animal offal and cereals. Both the energy content as well as ME distribution from protein, fat and carbohydrates were in accordance with the nutritional recommendations for adult foxes (Enggaard Hansen, 1992). Almost complete digestibility of dietary fat (>99%) was associated with the high quality of dietary fat (fish and poultry fat) and proves extremely high fat utilization in fur-bearing animals.

In the present study, digestibility coefficients for almost all individual FAs were over 97%. MUFA had higher digestibility as compared with their saturated counterparts (except for C24:0 and C24:1n-9), which coincides with the results of our earlier report on ileal digestibility of FAs in polar foxes (Burlikowska and Szymeczko, 2007). The greatest differences in MUFA and SFA digestibility were seen in case of long-chain FAs (C20 and C22). It is commonly known that FA chain length and unsaturation number influence fat absorption. In our study, especially MUFA of shorter chains had higher digestibility compared to long-chain MUFA. A similar trend was also observed in foxes fed different dietary fats when ileal and total digestibility was estimated (Burlikowska and Szymeczko, 2007). Generally, medium chain FAs are better absorbed than longer FAs because they can be solubilized in the aqueous phase of the intestinal contents, absorbed, bound to albumin and transported to the liver by the portal vein (Doreau and Chilliard, 1997; Ramirez *et al*., 2001).

Table 3: Apparent fat and fatty acid digestibility (mean±SEM) in blue foxes fed diets containing different levels of inulin

Item	Diet			
	0% Inulin	0.25% Inulin	0.5% Inulin	1% Inulin
Fat	99.59±0.05	99.57±0.06	99.42±0.07	99.45±0.04
C 14:0	97.75±0.34	98.37±0.20	98.06±0.29	98.09±0.19
C 15:0	97.93±0.19	98.10±0.34	97.02±0.52	97.68±0.20
C 16:0	99.68^{a}±0.04	99.68^{a}±0.04	99.53^{b}±0.05	99.51^{b}±0.05
C 17:0	99.20±0.09	99.01±0.17	98.66±0.15	98.82±0.12
C 18:0	99.19^{a}±0.11	99.19^{a}±0.09	98.81^{b}±0.11	98.81^{b}±0.11
C 20:0	78.85±2.50	84.99±2.08	81.02±2.74	79.96±1.29
C 21:0	99.55^{a}±0.11	98.76^{b}±0.20	98.57^{b}±0.26	99.04^{ab}±0.10
C 22:0	69.85^{ac}±2.34	73.61^{a}±3.84	62.10^{bc}±4.81	52.67^{b}±2.95
C 24:0	99.92±0.01	99.90±0.02	99.89±0.01	99.90±0.01
SFA	99.94±0.07	99.48±0.04	99.29±0.08	99.28±0.06
C 14:1n-5	99.93^{a}±0.03	99.77^{b}±0.04	99.74^{b}±0.05	99.84^{ab}±0.04
C 16:1n-7	99.84^{a}±0.02	99.82^{ac}±0.03	99.70^{b}±0.04	99.75^{bc}±0.02
C 18:1n-9 trans[1]	99.72 (99.52-99.80)	99.68 (99.24-99.82)	99.75 (99.12-99.93)	99.90 (99.87-99.92)
C 18:1n-9	99.78±0.03	99.71±0.08	99.60±0.05	99.64±0.03
C 20:1n-11	99.61^{a}±0.05	99.38^{ab}±0.12	99.11^{b}±0.11	99.26^{b}±0.07
C 22:1n-9	98.15^{a}±0.40	95.97^{b}±0.36	95.45^{b}±0.73	96.88^{ab}±0.45
C 24:1n-9	93.55±1.77	89.77±1.74	87.66±1.47	92.20±1.74
MUFA	99.78^{a}±0.03	99.78^{a}±0.03	99.63^{b}±0.05	99.68^{ab}±0.03
C 18:2n-6	98.95±0.16	99.16±0.26	98.49±0.19	98.54±0.14
C 20:3n-6	99.75^{a}±0.03	99.61^{ab}±0.08	99.44^{b}±0.07	99.54^{b}±0.04
C 20:4n-6	97.43^{a}±0.84	96.87^{a}±1.11	93.20^{b}±0.91	93.51^{b}±1.05
C 18:3n-3[1]	99.59 (95.93-99.84)	99.65 (82.58-99.85)	98.77 (98.25-99.64)	98.62 (96.87-99.83)
C 20:3n-3	99.18^{a}±0.26	98.49^{ab}±0.45	97.85^{b}±0.24	98.60^{ab}±0.13
PUFA	99.06±0.14	99.20±0.24	98.59±0.18	98.66±0.13
TFA	99.59±0.05	99.58±0.06	99.42±0.07	99.45±0.04

$^{a, b, c}$ Means in a row with different letters differ significantly (P<0.05); [1] Data are expressed as medians (range).

In the available literature, there is no information concerning the influence of inulin-type fructans on the digestibility of fat and FAs in foxes. Studies on different animal species (dogs, cats, rats and pigs) revealed that the effects of dietary fructans on nutrient digestibility are divergent and depend mainly on the level and form (fructan chain length and rate of fermentation) in which prebiotics are incorporated into the diet, on the administration method, as well as on the composition of a basal diet (Hesta *et al*., 2001; Flickinger *et al*., 2003; Barry *et al*., 2009; Krejpcio *et al*., 2009; Hedemann and Knudsen, 2010). In our experiment, relatively low concentrations (0.25, 0.5 and 1%) of inulin were used to minimalize the costs of diets and to avoid the adverse side-effects of fructans observed when higher supplementation was used (Diez *et al*., 1998; Hesta *et al*., 2001). Our study did not show any effect of inulin on the fat digestibility in blue foxes, which is in agreement with the results of some investigations on dogs, cats and rats (Propst *et al*., 2003; Krejpcio *et al*., 2009; Kanakupt *et al*., 2011). Lack of influence is probably due to the fact that inulin is almost completely degraded by colonic bacteria to soluble short chain FAs, thus does not interfere with absorption of fat from the gastrointestinal tract (Krejpcio *et al*., 2009). Contrary to our investigation, most experiments showed decline in fat digestibility (Diez *et al*., 1998; Hesta *et al*., 2001; Flickinger *et al*., 2003) and only a few revealed a positive effect of dietary inulin supplementation on nutrient digestibility (Barry *et al*., 2009; Hedemann and Knudsen, 2010).

No data are available on the influence of inulin on FA digestibility in monogastric animals. In the present study, the addition of inulin did not influence the digestibility of TFA, SFA and PUFA. Compared to the non-supplemented diet, MUFA digestibility was significantly lower in foxes fed with 0.5% inulin but the lower (0.25%) or higher inclusion (1%) had no effect. Generally, only small differences among mean values of digestibility coefficients for individual FAs were observed in our experiment. However, in some cases, the statistical analysis revealed significant differences (P<0.05), which was a result of extremely small intragroup variance. The most abundant dietary FA was palmitic acid (C16:0) that is generally the most common among SFA of animal diets (Nguyen *et al*., 2009). Decrease in digestibility of C16:0 and C18:0 in diets with 0.5 and 1% inulin could be indirectly related to the bifidogenic effect of inulin. In our previous study, the addition of 1% inulin resulted in a considerably larger number of lactic acid bacteria in the colon of polar foxes compared to the non-supplemented group (Marć-Pieńkowska *et al*., 2012). According to Hesta *et al*. (2001), lower apparent digestibility of crude fat observed in cats fed diets with inulin can be related with the fact that certain amounts of lipids are present in the membranes of large intestine bacteria. Furthermore, Hopkins *et al*. (2001) showed that the main cellular FAs of bacterial populations isolated from human feces were C16:0 and C18:0 which could, to some extent, explain the lower digestibility of these FAs determined in our experiment.

We did not find any influence of dietary inulin on PUFA digestibility. The two real main dietary EFAs for foxes are linoleic acid (LA) (C18:2n-6) and α-linolenic acid (ALA) (C18:3n-3) (NRC, 1982). Linoleic acid is generally thought to be responsible for maintaining the epidermal water barrier of the skin resulting in a healthy skin and well developed fur whereas ALA is a parent form of physiologically essential long-chain PUFA from n-3 family (NRC, 1982; Bauer, 2008; Case *et al*., 2011). In our study, the addition of inulin had no effect on LA and ALA digestibility which were 98.49 - 99.16 and 98.62 -99.65%, respectively. However, LA derivatives (C20:3n-6 and C 20:4n-6) had significantly lower digestibility in diets supplemented with 0.5 and 1% inulin compared to controls. Previous experiments on foxes fed diets with different levels and type of fat also showed high (generally exceeding 90%) total digestibility of LA and ALA (Rouvinen *et al*., 1988).

Conclusion: The present study demonstrates that in properly balanced diets covering maintenance requirement of adult blue foxes, fat is almost completely digested. Digestibility coefficients of fatty acids generally exceed 97% except for some of long-chain SFA. Low levels of dietary inulin have no effect on fat digestibility. Supplementation of 0.5 and 1% inulin decreases i.a. the digestibility of most abundant dietary SFA (C16:0 and C18:0) and derivatives of EFAs from n-6 family but has no effect on the absorption of their parent forms. Since the effect of inulin-type fructans on nutrient digestibility in carnivorous fur-bearing animals was not studied earlier, further investigations are needed to confirm the results of the present experiment.

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