Effect of fish oil supplementation and forage source on performance, rumen fermentation, nutrient digestion and chewing behaviour of Holstein young bulls

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Abstract:

BACKGROUND: Fat supplementation in ruminants ration often adversely affect dry matter intake (DMI), rumen fermentation and nutrients digestion. Basal diet ingredients is an undeniable determinant of ruminants responses including performance, chewing behaviour and nutrient digestibility to fat supplementation. **OBJECTIVES:** Current study was conducted to evaluate the effect of Fish oil (FO) supplementation under different proportion of alfalfa hay (AH) and corn silage (CS) in ration on performance, rumen fermentation, nutrient digestibility and chewing behaviour of Holstein young bulls. **METHODS:** Thirty -six Holstein young bulls were used in a 2 × 3 factorial arrangement, with 2 levels of AH proportion (10 and 20 % of AH versus 20 and 10 % of dietary CS) combined with 3 levels of FO supplement (0, 1 and 2.1% of dietary dry matter). Calves were fed TMR consisting of 30 % of forage and 70 % of barley grain-based concentrate mix (dry matter basis). The experiment lasted for 90 days. **RESULTS:** Dry matter intake was not affected by interaction of AH proportion and FO supplementation. Higher dietary CS proportion caused more DMI regardless of FO supplementation (8.71 versus 8.00 kg/d respectively for treatments with high and low CS proportion; $p< 0.01$). Highest level of FO reduced DMI regardless of AH proportion (8.65, 8.52 and 7.90 kg/d respectively for 0, 1 and 2.1 % of FO; $p< 0.01$). Rumen fermentation and nutrients digestibility were not affected by dietary treatments. Rumination times per kg of dry matter (DM) and physically effective neutral detergent fiber (peNDF) > 1.18 were increased in response to FO supplementation regardless of AH proportion ($p< 0.01$). High AH proportion showed lower total chewing activity and total rumination time ($p< 0.01$), higher chewing activity per kg of peNDF > 1.18 intake and eating times per kg of DM and peNDF > 1.18 intake $p< 0.01$) regardless of FO supplementation. **CONCLUSIONS:** The results demonstrate that AH can be replaced by CS to prevent depression in DMI and stimulate chewing activity in the case of fat supplementation.

Introduction

Lowered dry matter intake (DMI) and depressed nutrient digestibility are common consequences of fat supplementation in ruminant's diet and these deleterious effects can be exacerbated by enhancement in degree of unsaturation. Unsaturated FAs may decrease feed intake through decreasing the meal size or increasing inter-meal interval (Harvatine and Allen, 2006). Moreover, adding fat to ruminants rations may affect ruminal fiber digestion and passage rate, which can affect ruminal digesta pool size and chewing activity (Harvatine and Allen, 2005). The composition of basal diet has been reported to modulate the response to fat supplementation (Kargar et al., 2010). Fat supplementation in corn silage (CS) comparing with alfalfa hay (AH) based diets has been shown to negatively affect fiber digestion, rumen fermentation, DMI, and milk fat percentage in dairy cow (Onetti et al., 2001; Onetti et al., 2004). On the other hand, Kowsar et al. (2008) reported that replacing AH with CS in dairy cows diet can increase DMI and consumption of net energy for lactation (NEL) and neutral detergent fiber (NDF), however fiber digestion was decreased. Fiber is effective when it can be well digested in the rumen and in order to be well digested, it must be able to stimulate rumination, chewing activity and salivation (Beauchemin et al., 2008). Kowsar et al. (2008) reported that when diet is supplemented by unsaturated fat, a combination of ensiled and short cut hay forage simultaneously can increase DMI. Enhancement in particle size and subsequently physically effective NDF (peNDF) has been reported to increase mean retention time of lipid and promote its availability for microbial attack (Czerkawski and Clapperton, 1984) which can lessen antimicrobial behaviour of fat supplement. Hence, the objective of this study was to define an optimum CS to AH ratio to mitigate the likely decline in DMI and nutrient digestibility in response to fish oil (FO) supplementation through evaluation of feed intake, nutrients digestion and chewing behaviour of Holstein young bulls.

Materials and Methods

Animal, Housing and Diets: This experiment was conducted at the research dairy farm of Department of Animal Science, College of Agriculture and Natural Research, University of Tehran (Karaj- Iran). The study received approval from institutional Animal Care Committee. Thirty-six Holstein young bulls (with initial BW of 345±61 kg) were blocked by weight and randomly assigned to 6 dietary treatments following a 2 × 3 factorial arrangement (6 bulls in each treatment). Bulls were housed in individual pens. The experiment began after two weeks of an adaptation period to experimental diets and lasted for 90 days. Dietary treatments were 2 proportions of chopped AH (10 and 20 % of dietary dry matter basis) combined with 3 concentrations of FO (0, 1 and 2.1 % of dietary dry matter). The diets were balanced according to the nutrient requirement of NRC (1996) and were isocaloric and isonitrogenous and consisted of 30 % forage and 70 % concentrate mix (dry matter basis). Fish oil was added to concentrate and was prepared every 10 days to prevent fatty acid oxidation. The bulls were offered a total mixed ration (TMR) ad libitum and fed twice daily at 8:00 h and 17:00 h with approximately 5 to 10 % of refusals and had free access to water

and salt. The offered feed and refusals were recorded daily before the morning meal for calculation of DMI. Bulls were weighted monthly before the morning meal for calculation of average daily gain (ADG). Feed ingredients and diets were sampled every 2 weeks. Ingredients and chemical composition of diets are shown in Table 1.

Feed and fecal analysis: Samples of TMR and oats were collected and oven-dried at 60° C for 48 h every 2 weeks, ground to pass through 1-mm screen using a hammer mill (Arthur Hill Thomas Co., Philadelphia, PA) and stored until analyzed chemically. Fecal samples were obtained from the rectum for 1 week at the end of period and were oven-dried at 60 ºC for 48 h, ground and pooled for individual bull. Samples were analyzed for dry matter (DM; 945.15), ash (967.05), crude protein (CP; Kjeldahl N6.25,990.03) and ether extract (EE; 945.16) according to AOAC (1990). The NDF (without heat stable amylase) and acid detergent fiber (ADF) contents were analyzed according to Van Soest et al. (1991). The NDF and ADF fractions included the residual ash. Non-fiber carbohydrate (NFC) content was calculated by the following formula: NFC= 1000- (NDF g/kg + CP g/ kg + EE g/kg + ash g/kg). Acid-insoluble ash was used to calculate apparent digestibility of DM, organic matter (OM), CP, NDF and ADF according to Van Keulen and Young (1977).

Rumen fluid sampling and analysis: Rumen fluid samples were collected from each bull in the last week (d 83) at 0 and 4 h post feeding using a stomach tube attached to an Erlenmeyer flask connected to vacuum pump. To avoid saliva contamination, the initial 50 ml of rumen fluid was discarded and the pH of the second part was determined immediately using a portable pH meter (Sentron, model A102-003). Then, rumen fluid was filtered through a four layer cheese cloth and 25 ml of it was preserved by adding 5 ml of 25 % metaphosphoric acid solution for later determination of volatile fatty acids (VFA), and 20 ml was combined with 20 ml 0.2 N HCl for later measurement of ammonia nitrogen (NH3-N) concentration, and stored at -20 °C until analysis. Rumen fluid samples were thawed, centrifuged at 1200 RPM for 10 min, and the supernatant was analyzed for VFA by gas chromatography (Hewlett-Packard, model 5890, Avondale, PA) and determination of ruminal NH3-N concentration. Protozoa number in the rumen content was determined as described by Veira et al. (1983).

Particle size distribution: The Penn State Particle Separator (PSPS) was used to measure particles distribution of the TMR and forages. Samples of TMR and forage were obtained every 2 weeks and were used for PSPS analysis. After sieving, materials from each of the sieves were removed and dried at 55 °C to determine DM content. Physically effective NDF (peNDF) was estimated by multiplying dietary NDF percentage by the proportion of DM retained on the 19.0, 8.0, and 1.18 mm sieves of the PSPS (Kononoff et al., 2003).

Eating, ruminating and chewing activities: Eating and rumination activities were monitored visually for two periods of 48-h in the middle (d 44 and 45) and at the end of period (d 80 and 81). Eating and ruminating activities were recorded by 2 alternating individuals every 5 min and each activity was assumed to persist for the entire 5 min (Yang et al., 2000). Total time spent chewing was calculated as the time spent eating

plus the time spent ruminating.

Statistical analysis: Data were analysed by MIXED procedure of SAS (2002) in a 2 × 3 factorial arrangement. The mixed model included the fixed effects of AH, FO and their interaction and the random effect of animal. Least-square means were computed and tested for differences by the Tukey's test. The effect of increasing level of FO in the diet was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. The individual animal was considered as experimental unit. The difference between least-squared means was considered to be significant at $p< 0.05$.

Results

Performance: There was no interaction between AH and FO on DMI (Table 2). Inclusion of FO at 2.1 % level reduced DMI (8.65, 8.52 and 7.90 kg/d respectively for 0, 1 and 2.1 % of FO; $p< 0.01$) regardless of AH level. Treatments with higher proportion of CS showed higher DMI (8.71 versus 8.00 kg/d respectively for 10 and 20% of dietary AH; $p< 0.01$) regardless of FO supplementation. There was no interaction between AH and FO on growth performance including ADG and feed conversion ratio (FCR). Lower dietary AH proportion caused numerically higher FCR (8.13 versus 7.43 respectively for 10 and 20 % of dietary AH; $p< 0.11$) regardless of FO supplementation.

Nutrient digestibility: There were no differences in apparent digestibility of DM, OM, NDF, ADF, CP, NFC and EE affected by AH proportion, FO supplementation and their interaction (Table 3). Highest level of FO caused numerically higher EE digestibility (81.17, 80.98 and 87.76 % respectively for 0, 1 and 2.1 % of FO) regardless of AH proportion.

Rumen fermentation: Rumen fermentation parameters including total VFA, acetate, propionate, butyrate and acetate propionate ratio were not affected by interaction of AH proportion and FO supplementation (Table 4). There was an interaction of AH level and FO supplementation for rumen NH3-N concentration as addition of FO decreased rumen NH3-N concentration in a linear manner ($p< 0.01$) at lower proportion of AH, whereas NH3-N concentration increased quadratically ($p< 0.01$) at higher AH proportion. The second level of FO caused lower ruminal NH3-N content (8.71, 6.45 and 8.15 mg/dl respectively for 0, 1 and 2.1 % of FO; $p< 0.05$) regardless of dietary AH proportion. Moreover, higher proportion of dietary AH caused higher ruminal NH3-N content (6.97 versus 8.57 mg/dl respectively for 10 and 20% of dietary AH proportion; $p< 0.05$) regardless of FO supplementation. Second level of FO caused higher rumen pH (7.01, 7.40 and 7.15 respectively for 0, 1 and 2.1 % of FO; $p< 0.01$) and acetate to propionate ratio (3.19, 3.69 and 3.52 respectively for 0, 1 and 2.1 % of FO; $p< 0.11$) regardless of AH proportion, however, the difference was not statistically significant for acetate to propionate ratio. Second level of FO caused lower protozoa number regardless of AH proportion, however the difference was not statistically significant (26.00, 15.25 and 29.37 × 10^5/ml respectively for 0, 1 and 2.1 % of FO; $p< 0.06$).

Particle size distribution: Particle size distribution of CS, AH and TMR are presented in Table 5. The percentage of particles retained on top sieve (> 19 mm) was considerably higher in diets containing lower proportion of AH. The percentage of

Table 1. Ingredients and chemical composition of dietary treatments. FO: fish oil, AH: alfalfa hay, DM: dry matter, ME: metabolizable energy, CP: crude protein, NDF: neutral detergent fiber. 1. Each kg of vit and min premix contained: Ca 195 g; P 80 g; Mg 21 g; Na 50 g; Fe 3 g; Cu 0.3 g; Zn 0.3 g; Mn 22 g; I 0.12 g; Co 0.1 g; Se 0.02 g; vitamin A 600,000 IU; vitamin D 200,000 IU; vitamin E 200 IU.

	10%AH			20%AH		
%FO	0	1	2.1	0	1	2.1
Alfalfa hay	10	10	10	20	20	20
Corn silage	20	20	20	10	10	10
Barley grain	41	41	41	41	41	41
Wheat grain	2	1	0.5	5	3	0.5
Soybean meal	2	2	2	2	2	2
Canola meal	12	13	14	10	10	11
Beat pulp	5	5	5	5	5	5
Wheat bran	5	4	2	4	5	5
Zeolite	1	1	1	1	1	1
Calcium carbonate	0.5	0.55	0.68	0.4	0.5	0.62
Sodium bicarbonate	0.7	0.7	0.7	0.7	0.7	0.7
Vit and min premix1	0.7	0.7	0.7	0.7	0.7	0.7
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Fish oil	0	1	2.1	0	1	2.1
Chemical composition						
DM (%)	56	56	56	56	56	56
ME (Mcal/kg)	2.61	2.61	2.61	2.61	2.61	2.61
EE (% of DM)	3	4	5.1	3	4	5.1
CP (%)	15.5	15.5	15.5	15.5	15.5	15.5
NDF (%)	37	37	37	35	35	35

Table 2. Performance of Holstein young bulls fed different dietary fish oil level and alfalfa hay ratio. 1. AH, portion of alfalfa hay ratio; FO, effect of dietary fish oil level; AH×FO, fish oil × alfalfa hay interaction. FO: fish oil, AH: alfalfa hay, BW: body weight, ADG: average daily gain, DMI: dry matter intake, FCR: feed conversion ratio.

	10%AH			20%AH			p-value1				
%FO	0	1	2.1	0	1	2.1	SEM	AH	FO	AH×FO	FOL	FOQ
Initial BW	340	338	348	342	341	338	17.3	0.35	0.41	0.30	0.93	0.44
Final BW	448	447	452	447	447	436	5.2	0.19	0.70	0.26	0.89	0.74
ADG, kg/d	1.14	1.15	1.17	1.15	1.14	1.09	0.07	0.83	0.97	0.73	0.97	0.87
DMI, kg/d	9.15	8.70	8.29	8.16	8.34	7.51	0.25	0.01	0.01	0.45	0.61	0.26
FCR, kg/kg	8.71	8.34	7.34	7.63	7.60	7.07	0.48	0.11	0.18	0.76	0.69	0.53

particles retained on second (> 8.0 mm) and third sieves (> 1.18 mm) was higher in diets with lower AH proportion, however these differences were not statistically significant. Diets with greater proportion of AH had higher percentage of particles retained on bottom. These differences in particle size distribution caused higher percentage of peNDF > 1.18 mm in diet with higher proportion of CS.

Chewing behaviour: Chewing behaviour including eating, ruminating and total chewing times were not affected by interaction of AH proportion and FO supplementation except for total rumination time (Table 6). In treatments with low AH level, FO supplementation increased total rumination time per day, whereas in high AH level,

Table 3. Apparent nutrient digestibility of Holstein young bulls fed different dietary fish oil level and alfalfa hay ratio. 1. AH, portion of alfalfa hay ratio; FO, effect of dietary fish oil level; AH × FO, fish oil × alfalfa hay interaction. FO: fish oil, AH: alfalfa hay, DM: dry matter, OM: organic matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, CP: crude protein, NFC: non-fiber carbohydrate, EE: ether extract. 2. NFC= 1000 - (NDF g/kg + CP g/kg + ether extract g/kg + ash g/kg).

	10% AH			20% AH				p-value1				
%FO	0	1	2.1	0	1	2.1	SEM	AH	FO	AH×FO	FOL	FOQ
DM	87.17	85.57	86.85	88.74	86.00	87.02	2.04	0.67	0.54	0.93	0.27	0.34
OM	77.42	74.74	78.25	79.53	76.28	78.02	4.32	0.71	0.67	0.95	0.41	0.39
NDF	63.38	58.75	62.76	61.90	57.12	60.65	4.30	0.63	0.50	0.99	0.26	0.26
ADF	49.85	47.14	52.24	55.90	44.65	52.81	6.25	0.79	0.46	0.78	0.26	0.22
CP	77.65	72.94	79.09	77.88	74.53	79.12	3.41	0.83	0.28	0.96	0.23	0.12
NFC2	86.59	86.82	88.02	90.32	89.69	86.95	2.80	0.43	0.94	0.69	0.94	0.90
EE	86.30	79.98	88.12	76.03	81.97	87.41	3.06	0.24	0.07	0.11	0.94	0.19

Table 4. Rumen fermentation parameters of Holstein young bulls fed different dietary fish oil level and alfalfa hay ratio. 1. AH, portion of alfalfa hay ratio; FO, effect of dietary fish oil level; AH×FO, fish oil × alfalfa hay interaction. FO: fish oil, AH: alfalfa hay, VFA: volatile fatty acids, A/P: acetate to propionate ratio.

	10% AH			20% AH				p-value1				
%FO	0	1	2.1	0	1	2.1	SEM	AH	FO	AH×FO	FOL	FOQ
pH	7.04	7.41	7.21	6.97	7.40	7.09	0.08	0.33	0.01	0.76	0.01	0.01
NH3-N (mg/dl)	8.10	6.62	6.18	9.31	6.29	10.12	0.80	0.03	0.03	0.06	0.01	0.01
Acetate (mmol/dl)	60.32	62.85	57.91	56.75	59.52	59.95	4.13	0.63	0.80	0.73	0.53	0.51
Propionate (mmol/dl)	19.47	17.39	16.51	18.09	16.52	17.51	1.51	0.73	0.40	0.70	0.25	0.49
Butyrate (mmol/dl)	8.63	8.87	8.44	10.34	7.78	9.31	1.41	0.67	0.73	0.62	0.43	0.50
Total VFA (mmol/dl)	88.43	89.12	82.88	85.18	83.83	86.78	5.82	0.74	0.93	0.70	0.95	0.90
A/P	3.21	3.61	3.58	3.17	3.77	3.45	0.22	0.99	0.11	0.81	0.04	0.11
Protozoa, $\times 10^5$/ml	23.25	15.50	29.75	28.75	15	29	5.79	0.76	0.06	0.83	0.08	0.02

supplementation of FO reduced total rumination time per day ($p< 0.04$). The second level of FO caused lower total chewing activity (476.83, 432.92 and 468.12 min per day respectively for 0, 1 and 2.1 % of FO; $p< 0.07$), chewing activity per kg of DM intake (55.26, 48.78 and 57.53 min per kg of DM intake respectively for 0, 1 and 2.1 % of FO; $p< 0.01$) and chewing activity per kg of peNDF > 1.18 intake (209.62, 187.67 and 221.05 min per kg of peNDF intake respectively for 0, 1 and 2.1 % of FO; $p< 0.01$) regardless of AH proportion. Treatments with lower AH proportion showed higher total chewing activity (483.54 versus 435.04 min per day respectively for low and high AH proportion; $p< 0.01$) and lower chewing activity per kg of peNDF > 1.18 intake (198.19 versus 214.04 min per kg of peNDF > 1.18 intake respectively for low and high AH proportion; $p< 0.07$) regardless of FO supplementation. Moreover, total eating time (152.52, 132.19 and 136.88 min per day respectively for 0, 1 and 2.1 % of FO; $p< 0.01$), eating time per kg of DM intake (18.26, 15.00 and 16.86 min per kg of DM intake respectively for 0, 1 and 2.1 % of FO ; $p< 0.01$) and eating time per kg of peNDF > 1.18 intake (69.33, 57.82 and 65.16 min per kg of peNDF > 1.18 intake respectively for 0, 1 and 2.1 % of FO; $p< 0.05$) were decreased in a linear and quadratic manner in response to FO supplementation regardless of AH proportion. Eating times per kg

Table 5. Particle size distribution (% of total particles) and effective NDF content of alfalfa hay, CS, and TMR measured using the Penn State Particle Separator (PSPS). 1. AH, portion of alfalfa hay ratio; FO, effect of dietary fish oil level; AH×FO, fish oil × alfalfa hay interaction. FO: fish oil, AH: alfalfa hay, peNDF: physically effective neutral detergent fiber.

	10% AH			20% AH						p-value1		
%FO	0	1	2.1	0	1	2.1	Alfalfa hay	Corn silage	SEM	AH	FO	AH×FO
>19 mm	6.23	6.16	6.36	4.90	5.03	4.73	6.53	34.76	0.30	0.01	0.98	0.71
19.0-8.0 mm	15.46	15.13	14.53	15.73	15.23	15.76	28.43	56.40	0.31	0.05	0.31	0.18
8.0-1.18 mm	54.23	54.13	54.33	52.33	53.46	51.43	47.36	8.28	1.12	0.07	0.72	0.62
<1.18 mm	24.06	24.56	24.43	26.70	26.30	27.40	17.63	0.21	0.99	0.01	0.84	0.81
peNDF>1.18 mm	0.28	0.27	0.27	0.25	0.25	0.25	0.40	0.56	0.003	0.01	0.58	0.72

Table 6. Chewing behaviour of Holstein young bulls fed different dietary fish oil level and alfalfa hay ratio. 1. AH, portion of alfalfa hay ratio; FO, effect of dietary fish oil level; AH×FO, fish oil × alfalfa hay interaction. FO: fish oil, AH: alfalfa hay, peNDF: physically effective neutral detergent fiber. DMI: dry matter intake.

	10% AH			20% AH				p-value1				
%FO	0	1	2.1	0	1	2.1	SEM	AH	FO	AH×FO	FOL	FOQ
Chewing time												
Min/day	477.29	461.46	511.88	476.36	404.38	424.37	20.12	0.01	0.07	0.10	0.03	0.02
Min/kg DMI	53.33	50.49	58.62	57.19	47.08	56.24	2.16	0.77	0.01	0.37	0.02	0.01
Min/kg peNDF>1.18 intake	190.47	187.0	217.11	228.77	188.35	224.99	10.62	0.07	0.01	0.18	0.04	0.01
Eating time												
Min/day	152.08	135.63	134.79	152.95	128.75	138.96	6.64	0.91	0.01	0.69	0.01	0.03
Min/kg DMI	17.29	14.84	15.37	19.22	15.16	18.35	1.09	0.05	0.01	0.47	0.01	0.01
Min/kg peNDF>1.18 intake	61.78	54.97	56.93	76.88	60.66	73.40	4.25	0.01	0.02	0.38	0.01	0.01
Rumination time												
min/day	325.21	325.83	377.08	323.41	275.63	285.42	17.68	0.01	0.19	0.04	0.18	0.07
Min/kg DMI	36.03	35.64	43.24	37.97	31.91	37.89	2.19	0.18	0.01	0.22	0.14	0.01
Min/kg peNDF>1.18 intake	128.69	132.02	160.18	151.88	127.68	151.59	8.43	0.62	0.01	0.13	0.22	0.01

of DM intake (15.83 versus 17.58 min per kg of DM intake respectively for 10 and 20% of dietary AH; $p< 0.05$) and peNDF > 1.18 intake (57.89 versus 70.32 min per kg of peNDF > 1.18 intake respectively for 10 and 20% of dietary AH; $p< 0.01$) were higher in treatments with higher proportion of AH regardless of FO supplementation. Rumination times per kg of DM intake (37.00, 33.78 and 40.57 min per kg of DM intake respectively for 0, 1 and 2.1% of FO; $p< 0.01$) and peNDF > 1.18 intake (140.29, 129.85 and 155.88 min per kg of peNDF > 1.18 intake respectively for 0, 1 and 2.1% of FO; $p< 0.01$) were increased quadratically with addition of FO regardless of AH proportion. The total rumination time per day was significantly higher in treatments with lower proportion of AH (342.17 versus 294.82 min per day respectively for 10 and 20% of dietary AH; $p< 0.01$) regardless of FO supplementation.

Discussion

Performance: Reduced DMI as a consequence of FO supplementation is in agreement with Wistuba et al. (2006) and Aliza-

deh et al. (2012) who reported lowered DMI in response to FO supplementation in the diet of steers and dairy cows respectively. Dietary lipid (> 8 %) has been reported to affect DMI detrimentally due to negative effects of lipids, especially PUFA on rumen microbial activity (Nawaz and Ali, 2016). Moreover, depressed DMI in response to fat supplementation might be mediated through changes in chemical mediators such as cholecystokinin, glucagon-like peptide-1 and/or intestinal peptides (Martínez Marín et al., 2013). In the present study, reduction in DMI in response to FO supplementation can be related to its palatability rather than its deleterious effects on rumen fermentation and nutrients digestion as none of these parameters were affected by FO supplementation which is in agreement with (Kadkhoday et al., 2017). Higher DMI in treatments with higher proportion of CS in current study is in agreement with Kowsar et al. (2008) who reported increased DMI when AH was partially replaced by CS. The deleterious effect of fat supplementation on DMI has been reported to be more when CS is the main forage source of the diet (Martínez Marín et al., 2013) which is not in accordance with current study. Higher DMI in treatments with higher CS proportion in current study can be attributed to higher moisture and palatability of diets containing CS (Kowsar et al., 2008). In current study, although water was added exactly before offering TMR to equalize the dietary DM content among treatments, it was absorbed to the surface of the particles and was not that effective for maintaining the uniformity of diets containing higher proportion of AH. On the other hand, Onetti et al. (2004) eliminated the decline in DMI of dairy cow fed tallow supplemented diet through replacing CS with AH. Moreover, by increasing the alfalfa silage: CS ratio, Onetti et al. (2002) was successful to alleviate the detrimental effect of tallow supplementation on DMI of dairy cows. These differences in response seems to originate from the shape and quality of the AH they used in their study. In current study, the decreased DMI without affecting the growth performance and FCR is in agreement with Wistuba et al. (2006), however, numerically improved FCR in treatments with higher proportion of AH can be related to the fact that higher proportion of AH decreased DMI without adverse effect on final BW and ADG. The results indicate that increasing FO level in a high concentrate diet has limited effects on improving growth rate and feed efficiency.

Nutrient digestibility: In the study of Kowsar et al. (2008), digestibility of DM, OM and ADF decreased as AH was replaced by CS in fat supplemented diet of dairy cow, however digestibility of NDF and CP was not affected. They related this depression to higher consumption and lower chewing activity per kg of peNDF > 1.18 in diet with higher proportion of CS. Salem et al. (1993) reported a reduction in fiber digestion when rapeseed oil (7% of dietary DM) was supplemented to CS-based diet, whereas this depression was not observed in hay-based diet. In present study, although diets with higher proportion of CS increased DMI, these diets also increased total chewing and rumination activity compared to the diets with higher AH level. Hence, there was no difference in rumination times per kg of DM and peNDF > 1.18 intake between treatments with low and high AH proportion. In addition, the level of fat supplementation in current study was far lower than those studies (Salem et al., 1993; Mach et al., 2006)

which reported depressed fiber digestion in response to fat supplementation. The numerical enhancement in EE digestibility in response to enhancement in level of FO is in agreement with the study of Pirondini et al. (2015) in which FO supplementation in dairy cows ration increased EE digestibility. They attributed higher EE digestibility of FO supplemented diets to almost complete digestion of FO lipids. Moreover, it has been reported that total EE digestibility increases by enhancement in level of fat supplementation (Patra, 2013).

Rumen fermentation parameters: Reduced rumen NH3-N concentration in second level of FO is in agreement with Bhatt et al. (2011) who reported lowered NH3-N concentration in rumen fluid of lambs in response to fat supplementation. The deleterious effect of fat supplementation on rumen NH3-N content has been reported to be related to decreased number of protozoa and depressed recycling of ruminal microbial nitrogen (Onetti et al., 2001). In current study, the lowered NH3-N content in second level of FO coincided with lowered number of protozoa which is in agreement with (Bhatt et al., 2011). There is no clear explanation for lowered rumen NH3-N content and protozoa number in second but not third level of FO. Lowered DMI and possibly lowered passage rate in third but not second level of FO can be the likely explanation for lack of reduction in NH3-N content and protozoa number in third level of FO. Higher rumen NH3-N content in treatments with higher proportion of AH might be related to higher protein and non-protein nitrogen (NPN) content of AH comparing to CS. Replacing CS by alfalfa silage has been reported to increase the concentration of rumen NH3-N in fat supplemented diet of dairy cow which was attributed to high NPN content of alfalfa silage and extensive ruminal degradation of its protein (Onetti et al., 2002). The lowered rumen NH3-N content in response to FO supplementation in treatments with higher CS but not AH proportion can be related to lower NPN content of CS compared to AH and also higher coating effects of FO on CS compared to AH (Smith et al., 1993). Mach et al. (2006) reported a significant decline in ruminal VFA concentration and enhancement in rumen pH when whole canola and linseed (5, 8 and 11 % of dietary DM) were supplemented to Holstein bulls diet which was attributed to reduction in dietary NFC content as a result of fat supplementation. In current study, although there was no difference in VFAs concentration among dietary treatments, higher rumen pH in FO supplemented diets is in accordance with numerically higher acetate to propionate ratio in these treatments. Kowsar et al. (2008) observed no difference in molar concentration of VFAs among treatments with various proportion of AH and CS supplemented by fat, whereas Onetti et al. (2002) reported increased ruminal VFA production through replacing CS with AH and this replacement increased and decreased, respectively, the concentrations of acetate and propionate which subsequently increased acetate to propionate ratio. Kowsar et al. (2008) reported a linear increase in rumen pH through enhancement in dietary proportion of CS compared to AH, which was attributed to higher chewing activity as a result of higher peNDF intake and subsequently a stabilized rumen environment in treatments with higher proportion of CS. In present study, the reasons why these parameters were not affected by dietary treatment can be due to lower level of fat supplementation

and lower dietary forage percentage (30 %). The decreased protozoa number in second level of FO in current study is in agreement with Bhatt et al. (2011) who supplemented coconut oil in lambs diet, however, Kirovski et al. (2015) reported enhanced protozoa number as dairy cows received diets supplemented with palm oil and Messana et al. (2012) observed no change in protozoa count as a consequence of enhancement in lipid content of bulls diet. In addition to fat supplementation, the level of DMI seems to be a prerequisite for protozoa number to be influenced. In current study, lowered DMI can be a possible explanation for higher protozoa number in higher level of FO as lower DMI decreases passage rate which can lead to higher persistency of protozoa in the rumen.

Chewing behaviour: The lowered total chewing activity, chewing activity per kg of DM and peNDF intake in second level of FO are in agreement with Onetti et al. (2004) who reported increased time for chewing and rumination per kg of DM intake and rumination per kg of NDF intake when tallow was supplemented dairy cows diet. The lowered total eating time and eating times per kg of DM and peNDF in response to FO supplementation in current study are not in agreement with the study of Kargar et al. (2010) in which eating rate was lowered in dairy cows fed diet supplemented with yellow grease. The reduction in eating times per kg of DM and peNDF intake in addition to reduction in total eating time demonstrates that the relative reduction in total eating time was more than reduction in DMI in response to FO supplementation. This implies that despite the lowered DMI as a consequence of FO supplementation, bulls tried to consume in higher rate, possibly to fulfil their requirement. Increased rumination time per kg of DM and peNDF > 1.18 intake as a consequence of FO supplementation can be related to coating effect of supplemented fat on particles as fat supplementation has been reported to lower fiber digestion (Nawaz and Ali, 2016) and depressed fiber digestion may decrease the rate of reduction in particle size during chewing activity through decreasing tissue fragility (Harvatine and Allen, 2006), hence more rumination would be required (Onetti et al., 2004). Smith et al. (1993) reported that ruminal fermentation is inhibited more when fat is supplemented to CS based compared to AH based diets due to more accessibility of CS to coating effect of lipid. Hence, higher total rumination time in response to FO supplementation in treatments with high CS proportion in current study was in fact a strategy to overcome this limitation and can be beneficial in preventing depression in fiber digestibility. Moreover, increased chewing time per kg of peNDF > 1.18 intake in diets with higher proportion of AH can be explained by higher eating time per kg of peNDF > 1.18 intake probably due to lower palatability of AH compared to CS containing diets. Lower eating times per kg of DM and peNDF > 1.18 intake in treatments with higher proportion of CS is in agreement with Kowsar et al.'s study (2008) in which replacing AH with CS increased total eating time and reduced eating time per kg of physically effective fiber (PEF) intake, however eating time per kg of DM intake was not affected. In their study, higher rate of eating for diet containing higher proportion of CS was attributed to more palatability of CS comparing to AH and also the particle size of AH. In present study, although treatments with higher

proportion of CS provided more peNDF > 1.18, eating time per kg of DM and peNDF > 1.18 intake was lower in diets with higher proportion of CS which verifies the higher palatability of CS containing diets. Reduced total chewing time in treatments with higher AH proportion might be due to reduction in total rumination time as total eating time did not show any difference between low and high AH containing treatments. The lack of difference in total eating time between treatments with low and high AH proportion despite higher eating times per kg of DM and peNDF > 1.18 intake in treatments with high AH proportion can be related to reduced DMI due to lower palatability in diets containing high AH proportion. The enhanced chewing time per kg of peNDF > 1.18 intake in treatments with higher proportion of AH was due to enhanced eating time per kg of peNDF > 1.18 as there was no difference in rumination time per kg of peNDF > 1.18 between treatments with low and high AH proportion.

Conclusion: Increasing substitution of AH for CS from 10 to 20% and increasing FO supplementation up to 2.1% of dietary DM in finishing diets decreased DMI but did not affect growth performance and feed efficiency. As inclusion of CS to diet reduced time of eating per kg of DM and peNDF >1.18 intake and also diets with higher proportion of CS showed higher DMI, CS can be used in fat supplemented diets to alleviate the likely reduction in DMI. Moreover, since higher proportion of CS in diet increased total time of rumination, using CS in fat supplemented diets can prevent the likely deleterious effects of fat supplementation on rumen fermentation and nutrient digestibility.

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References

Alizadeh, A., Alikhani, M., Ghorbani, G., Rahmani, H., Rashidi, L., Loor, J. (2012) Effects of feeding roasted safflower seeds (variety IL-111) and fish oil on dry matter intake, performance and milk fatty acid profiles in dairy cattle. J Anim Physiol Anim Nutr. 96: 466-473.

AOAC, W.H. (1990) Official methods of analysis. Arlington, VA,USA.

Beauchemin, K., Eriksen, L., Nørgaard, P., Rode, L. (2008) Short communication: Salivary secretion during meals in lactating dairy cattle. J Dairy Sci. 91: 2077-2081.

Bhatt, R., Soren, N., Tripathi, M., Karim, S. (2011) Effects of different levels of coconut oil supplementation on performance, digestibility, rumen fermentation and carcass traits of Malpura lambs. Anim Feed Sci Technol. 164: 29-37.

Council, N.R. (1996) Nutrient requirements of beef cattle, National Academy Press Washington, DC, USA.

Czerkawski, J., Clapperton, J. (1984) Fats as energy-yielding compounds in the ruminant diet. In: Fats in animal nutrition. Wiseman, J. (ed.). Bullerwo rth, Londonm, Uk.

Harvatine, K., Allen, M. (2005) The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J Dairy Sci. 88: 4018-4027.

Harvatine, K., Allen, M. (2006) Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. J Dairy Sci. 89: 1104-1112.

Kadkhoday, A., Riasi, A., Alikhani, M., Dehghan-Banadaky, M., Kowsar, R. (2017) Effects of fat sources and dietary C18: 2 to C18: 3 fatty acids ratio on growth performance, ruminal fermentation and some blood components of Holstein calves. Livest Sci. 204: 71-77.

Kargar, S., Khorvash, M., Ghorbani, G., Alikhani, M., Yang, W. (2010) Effects of dietary fat supplements and forage: concentrate ratio on feed intake, feeding, and chewing behavior of Holstein dairy cows. J Dairy Sci. 93: 4297-4301.

Kirovski, D., Blond, B., Katić, M., Marković, R., Šefer, D. (2015) Milk yield and composition, body condition, rumen characteristics, and blood metabolites of dairy cows fed diet supplemented with palm oil. Chem Biol Technol Agric. 2: 6.

Kononoff, P., Heinrichs, A., Buckmaster, D. (2003) Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. J Dairy Sci. 86: 1858-1863.

Kowsar, R., Ghorbani, G., Alikhani, M., Khorvash, M., Nikkhah, A. (2008) Corn silage partially replacing short alfalfa hay to optimize forage use in total mixed rations for lactating cows. J Dairy Sci. 91: 4755-4764.

Mach, N., Devant, M., Díaz, I., Font-Furnols, M., Oliver, M., García, J., Bach, A. (2006) Increasing the amount of n-3 fatty acid in meat from young Holstein bulls through nutrition. J Anim Sci. 84: 3039-3048.

Martínez Marín, A.L., Pérez Hernández, M., Alba, P., Luis, M., Carrión Pardo, D., Garzón Sigler, A.I., Gómez Castro, G. (2013) Fat addition in the diet of dairy ruminants and its effects on productive parameters. Rev Colomb Cienc Pec. 26: 69-78.

Messana, J.D., Berchielli, T.T., Arcuri, P.B., Ribeiro, A.F., Fiorentini, G., Canesin, R.C. (2012) Effects of different lipid levels on protozoa population, microbial protein synthesis and rumen degradability in cattle. Acta Sci Anim Sci. 34: 279-285.

Nawaz, H., Ali, M. (2016) Effect of supplemental fat on dry matter intake, nutrient digestibility, milk yield and milk composition of ruminants. Pak J Agr Sci. 53: 271-275.

Onetti, S., Reynal, S., Grummer, R. (2004) Effect of alfalfa forage preservation method and particle length on performance of dairy cows fed corn silage-based diets and tallow. J Dairy Sci. 87: 652-664.

Onetti, S., Shaver, R., McGuire, M., Grummer, R. (2001) Effect of type and level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. J Dairy Sci. 84: 2751-2759.

Onetti, S., Shaver, R., McGuire, M., Palmquist, D., Grummer, R. (2002) Effect of supplemental tallow on performance of dairy cows fed diets with different corn silage: alfalfa silage ratios. J Dairy Sci. 85: 632-641.

Patra, A.K. (2013) The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. Livest Sci. 155: 244-254.

Pirondini, M., Colombini, S., Mele, M., Malagutti, L., Rapetti, L., Galassi, G., Crovetto, G. (2015) Effect of dietary starch concentration and fish oil supplementation on milk yield and composition, diet digestibility, and methane emissions in lactating dairy cows. J Dairy Sci. 98: 357-372.

Salem, H.B., Krzeminski, R., Ferlay, A., Doreau, M. (1993) Effect of lipid supply on in vivo digestion in cows: comparison of hay and corn silage diets. Can J Anim Sci. 73: 547-557.

Smith, W., Harris, B., Van Horn, H., Wilcox, C. (1993) Effects of Forage Type on Production of Dairy Cows Supplemented with Whole

Cottonseed, Tallow, and Yeast1. J Dairy Sci. 76: 205-215.

Van Keulen, J., Young, B. (1977) Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J Anim Sci. 44: 282-287.

Van Soest, P.V., Robertson, J., Lewis, B. (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74: 3583-3597.

Veira, D., Ivan, M., Jui, P.Y. (1983) Rumen ciliate protozoa: effects on digestion in the stomach of sheep. J Dairy Sci. 66: 1015-1022.

Wistuba, T., Kegley, E., Apple, J. (2006) Influence of fish oil in finishing diets on growth performance, carcass characteristics, and sensory evaluation of cattle. J Anim Sci. 84: 902-909.

Yang, W., Beauchemin, K., Rode, L. (2000) Effects of barley grain processing on extent of digestion and milk production of lactating cows. J Dairy Sci. 83: 554-568.

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اثر مکمل کردن روغن ماهی و نوع علوفه بر عملکرد، تخمیر شکمبه‌ای، هضم مواد مغذی و رفتار جویدن گوساله‌های نر هلشتاین

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چکیده

زمینه مطالعه: مکمل کردن چربی در جیره نشخوارکنندگان اغلب دارای اثرات منفی بر مصرف ماده خشک، تخمیر شکمبه‌ای و قابلیت هضم مواد مغذی می‌باشد. ترکیب جیره پایه یکی از عوامل تعیین‌کننده غیر قابل انکار در پاسخ نشخوارکنندگان شامل عملکرد، رفتار جویدن و قابلیت هضم مواد مغذی به مکمل کردن چربی به جیره می‌باشد. **هدف:** مطالعه حاضر به منظور بررسی اثر مکمل کردن روغن ماهی به جیره‌های با نسبت‌های متفاوت ذرت سیلو شده و یونجه خشک بر عملکرد، تخمیر شکمبه‌ای، قابلیت هضم مواد مغذی و رفتار جویدن گوساله‌های نر هلشتاین بوده است. **روش کار:** تعداد ۳۶ راس گوساله نر هلشتاین در قالب یک طرح فاکتوریل ۲ × ۳ با ۲ سطح علوفه یونجه (۱۰ و ۲۰ ٪ ماده خشک جیره) و ۳ سطح مکمل روغن ماهی (۰، ۱ و ۲/۱ ٪ ماده خشک جیره) مورد استفاده قرار گرفت. گوساله‌ها جیره کاملاً مخلوط حاوی ۳۰ ٪ علوفه (نسبت‌های متغییر ۱۰ و ۲۰ ٪ علوفه خشک یونجه و ذرت سیلو شده) و ۷۰٪ کنسانتره بر پایه جو (بر اساس ماده خشک) را دریافت کردند. طول مدت آزمایش ۹۰ روز بود. **نتایج:** مصرف ماده خشک تحت تأثیر اثر متقابل نسبت یونجه خشک و مکمل روغن ماهی قرار نگرفت. نسبت بالاتر ذرت سیلو شده در جیره صرف نظر از سطح مکمل روغن ماهی باعث افزایش ماده خشک مصرفی شد (۸/۷۱ در برابر ۸/۰۰ kg در روز به ترتیب برای تیمارهای با سطوح پایین و بالای یونجه خشک در جیره: $p>0/01$). بالاترین سطح روغن ماهی باعث کاهش ماده خشک مصرفی صرف نظر از نسبت یونجه خشک در جیره شد (۸/۶۵ kg، ۸/۵۲ و ۷/۹۰ در روز به ترتیب برای سطوح ۰، ۱ و ۲/۱ ٪ روغن ماهی در جیره: $p>0/01$). تخمیر شکمبه‌ای و قابلیت هضم مواد مغذی تحت تأثیر تیمارهای تغذیه‌ای قرار نگرفتند. زمان‌های نشخوار به ازای کیلوگرم ماده خشک و کیلوگرم peNDF < ۱/۱۸ در پاسخ به مکمل کردن روغن ماهی و صرف نظر از نسبت یونجه خشک در جیره افزایش یافتند ($p>0/01$). نسبت بالای یونجه خشک در جیره باعث کاهش کل زمان جویدن و کل زمان نشخوار در روز ($p>0/01$)، افزایش زمان جویدن به ازای کیلوگرم peNDF < ۱/۱۸ مصرفی و زمان خوردن به ازای کیلوگرم ماده خشک و کیلوگرم peNDF < ۱/۱۸ مصرفی ($p>0/01$) صرف نظر از سطح مکمل روفن ماهر در جیره شد. **نتیجه‌گیری نهایی:** نتایج مطالعه حاضر نشان می‌دهد که یونجه خشک می‌تواند به منظور جلوگیری از کاهش در ماده خشک مصرفی و همچنین تحریک فعالیت جویدن در شرایطی که چربی به جیره مکمل می‌شود، توسط ذرت سیلو شده جایگزین گردد.

واژه‌های کلیدی: فعالیت جویدن، مکمل کردن چربی، نوع علوفه، قابلیت هضم مواد مغذی، عملکرد

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