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EFFECTS OF CONCENTRATE LEVELS AND OIL SUPPLEMENTATION IN DIETS ON RUMEN CHARACTERISTICS AND PERFORMANCE OF CATTLE

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ABSTRACT

This study investigated the effects of concentrate levels and oil supplementation on rumen characteristics and growth performance of cattle. Twenty-four Brahman crossbred bulls were arranged in a 2x3 factorial design with two levels of concentrate (0.5 and 1.5% body weight) and three supplemental sources (60 g soybean oil/kg dry matter (DM), 60 g fish oil/kg DM and non-oil). Total rumen bacteria number was higher in cattle fed diets with lower concentrate level ($P \leq 0.05$), yet it was not affected by oil supplementation. Acetic and butyric acid concentration in rumen decreased with the increase of concentrate level in diet. The intake of total DM and crude protein were higher in cattle fed higher concentrate diet (6.25 kg/day and 778 g/day, respectively). In terms of growth performance, the higher concentrate level cattle consumed, the better weight gain was achieved (768 g/day). Soybean oil addition helped attain the highest weight gain of 738 g/day, while similar weight increase was observed in cattle fed non-oil and fish oil supplemented diets (624 g/day and 641 g/day, respectively). Supplementation of concentrate and oil also increased weight gain and the maximum daily weight gain (851 g/day) was recorded in cattle fed the diet containing soybean oil combined with 1.5% concentrate.

Keywords: cattle; concentrate; fish oil; soybean oil; weight gain; rumen fermentation

INTRODUCTION

In Vietnam, cattle are often fed grass-based diet combined with rice straw and rice bran. These feed sources are relatively cheap and readily available, but cattle growing time is considerably long and the daily gain is usually lower than expected. Therefore, it is important to find supplemental feeds to improve cattle growth rate. Concentrate is a nutrient-balanced diet that is easily fermented, hence it stimulates microbial growth in the rumen and this in turn accelerates the microbial digestion and increases feed consumption and weight gain. The addition of concentrate to diet has received considerable attention from many researchers (Ba *et al.*, 2008; Doyle *et al.*, 2008; Moletta *et al.*, 2014) but the results of these studies varied greatly depending on cattle breeds and levels of supplements. For example, a feeding rate of 2.2% live weight was suggested for local cattle under fattening period (Dung *et al.*, 2013) while in cross-bred Brahman cattle at 11-12 months of age, the recommended concentrate level was 1.2% body weight instead of 1.8% or 2.4% of body weight, to which the growth rate responded slowly (Quang *et al.*, 2015). The use of concentrate in the diet is also known to contribute to an improvement in organic matter digestibility, increased nitrogen accumulation (Wanapat and Khampa, 2007) and thus it can shorten fattening time and increase profits.

In addition to concentrate, the effects of oil supplementation to beef cattle diet have been widely studied (Ueda *et al.*, 2003; Yang *et al.*, 2009). The impact of lipid supplementation on the ruminal fermentation is based primarily on oil source (Wachira *et al.*, 2000), oil level (Shingfield *et al.*, 2008), and dietary forage: concentrate ratio (Ueda *et al.*, 2003). Soybean oil is rich in unsaturated fatty acids, namely linoleic acid and α-linolenicacid that are often included in the ruminant diet to increase meat production (Harfoot and Hazlewood, 1997). Zinn (1989) also found that supplementing up to 8% oil improved the growth and feed efficiency of the fattened cattle. Moreover, fish oil is known by its high content of eicosapentaenoic acid and docosahexaenoic acid that can help improve fiber digestibility (Doreau and Chilliard, 1997).

The combination of concentrate with vegetable oil or fish oil has certain effects on rumen characteristics and microoganisms. In sheep, Gómez-Cortés *et al.* (2008) showed that rumen fermentation was not affected by a concentrate-rich diet containing 6 g/kg DM of soybean oil. Previously, Palmquist (1988) also indicated that the addition of unsaturated oil to the diet would have unfavorable effects on DM absorption and fermentation in rumen. This negative effect can be reduced if the diet contains higher proportion of forage. However, the amount of information concerning this aspect of cattle remains limited. Therefore, the present study was carried out to determine the effects of concentrate levels with or without the supplementation of soybean oil and fish oil on rumen characteristics and growth performance of Brahman crossbred cattle.

MATERIALS AND METHODS

Animal, diet and experimental design: Twenty-four Brahman crossbred bulls (11-12 months old with 189.3±11.2 kg live weight) were arranged in a 2x3 factorial design with two levels of commercial concentrate (0.5% and 1.5% body weight) and three supplemental sources (60 g soybean oil/kg DM, 60 g fish oil/kg DM and no supplement). Chemical composition of the ingredients and diets is presented in Table 1 and Table 2. Hymenachne grass *(Hymenachne acutigluma)* (approximately 1% of body weight, DM basis) was divided into two parts for daily feeding at 08:00 and 14:00. Rice straw was given freely in the evening. Soybean and fish oil were divided and mixed with concentrate in separate troughs and were fed to the animals twice a day before grass feeding. All cattle were given the same basic diet 15 days before the start of 90-day experiment.

Table 1. Chemical composition of ingredients used in diets.

Item	*Hymenachne acutigluma* grass	Rice straw	Concentrate	Soybean oil	Fish oil
Dry matter (%)	18.9	91.8	86.0	-	-
Organic matter (% DM)	90.1	87.1	92.7	-	-
Crude protein (% DM)	10.3	4.6	16.0	-	-
Neutral detergent fiber (% DM)	64.6	74.2	26.9	-	-
Acid detergent fiber (% DM)	32.5	42.0	10.6	-	-
Metabolizable energy (MJ/kg DM)*	9.4	5.7	10.3	36.8	37.7

*Values were calculated from composition and nutritive values of animal feeds in Vietnam according to National Institute of Animal Husbandry (2000).

Sampling: Cattle were weighed on two consecutive days in the morning before feeding at the beginning of the experiment, after 30, 60 and 90 days. A digestion trial was performed during the last 7 days of the experiment. In this period, all feces were collected, weighed and stored in a freezer at -20°C for analysis. Feed offered and residues were recorded daily and analyzed for the chemical composition to calculate digestibility. At the end of the trial, rumen fluid was collected through a stomach tube before feeding. About 200 ml of rumen fluid was used for immediate pH measurement using a Delta-320 pH meter (Mettler Toledo, USA). The rest of rumen fluid samples were filtered through 4 layers of cheesecloth and stored at -80°C for DNA extraction.

Table 2. Nutrient composition of different diets.

Diets	No oil		Soybean oil		Fish oil	
	0.5% concentrate	1.5% concentrate	0.5% concentrate	1.5% concentrate	0.5% concentrate	1.5% concentrate
Ingredients (%)						
Concentrate	18.4	48.7	18.4	47.0	18.5	48.9
Grass	36.8	32.5	36.8	31.3	36.9	32.6
Oil	-	-	6.0	6.0	6.0	6.0
Rice straw	44.9	18.8	38.9	15.6	38.6	12.6
Chemical composition of diets (%)						
DM	63.9	65.3	64.4	66.7	64.3	65.7
CP	8.8	12.0	8.5	11.5	8.5	11.8
NDF	62.0	48.0	57.5	44.5	57.5	43.5
ADF	32.7	23.6	30.2	21.7	30.2	21.0
ME, MJ/Kg DM	22.3	47.3	24.2	47.7	24.3	49.3

Sample analysis: Feed and feces samples were dried at 60°C and ground by a Retsch grinder (Retsch, Germany) for further analysis (AOAC, 1990). Nitrogen (N) determination was done using Macro Kjeldahl method and crude protein (CP) content was calculated as N×6.25. Neutral detergent fiber (NDF) and acid detegent fiber (ADF) were analyzed by the procedure of Van Soest *et al.* (1991). The concentration of ammonia nitrogen (NH_3-N) was determined by diluting 10 g of ruminal fluid in 50 ml distilled water, followed by distillation of the released ammonia by Kjeldahl method. The volatile fatty acids

(VFA) in the rumen fluid were analyzed by using High Pressure Liquid Chromatography (Mathew *et al.*, 1997).

Quantification of rumen bacteria by real-time PCR: Rumen samples from the same cattle were pooled for DNA extraction. Microbe genomic DNA was extracted by using CTAB method (Minas *et al.*, 2011). The primers and thermal conditions used in PCR and real-time PCR were taken from Denman and McSweeney (2006) for total bacteria and from Koike and Kobayashi (2001) for *Ruminococcusalbus*, *Fibrobactersuccinogenes* and *Ruminococcusflavefaciens* quantification. The purified PCR products were cloned in TOPO® TA Cloning® Kits (Invitrogen) and the process was carried out in accordance with the instructions of the manufacturer. The recombinant plasmids were then extracted by the PureLink® Quick Plasmid Miniprep Kits (Invitrogen). DNA plasmid was diluted with concentration from 10^8 to 10^1 to generate standard curves separately for each type of bacteria. The number of DNA copy was calculated based on threshold cycle (C_T) value and via dissociation curve analysis (Petri *et al.*, 2013).

Statistical analysis: The analysis of variance was performed using the General Linear Model procedure of Minitab software version 16.2.1 (Minitab, 2010). Differences between dietary treatments were determined by using Tukey's pairwise comparisons ($P \leq 0.05$).

RESULTS

The concentration of NH_3-N in cattle rumen was not significantly different at various treatments of concentrate levels and oil sources in the diets. Moreover, different sources of oil supplements had significant effect on rumen pH, and the lowest pH was found in rumen of cattle having 1.5% concentrate (Table 3). The number of total bacteria in cattle fed 0.5% concentrate was more than two times higher than that in cattle having 1.5% concentrate. However, different oil sources did not significanty affect bacterial population. The number of cellulytic bacteria was lower at higher percentage of concentrate in diet. *F. succinogenes* appeared to be lowest in cattle supplemented with fish oil (3.72×10^{10} copies mL^{-1}, $P \leq 0.05$). With the increase of concetrate level in diet, the concentration of acetic and butyric acid decreased while that of propionic acid remained unchanged (Table 3). Acetic and propionic acid concentrations in rumen of cattle were the highest in non-oil treatment. Significantly higher acetate:propionate ratio in rumen VFAs was obsreved in 0.5% concentrate treatment. No interaction of concentrate level and oil supplementation was found for the investigated rumen parameters.

Table 3. Effects of concentrate levels and oil supplementation on rumen characteristics of cattle.

Items	Concentrate levels		SEM	Oil sources			SEM	*p* value		
	0.5%	1.5%		No oil	Soybean oil	Fish oil		C	O	C x O
pH	6.90	6.73	0.03	6.78	6.85	6.82	0.03	0.001	0.365	0.367
NH_3 (mg/L)	159.0	151.9	7.66	152.2	145.7	168.5	9.38	0.524	0.247	0.520
Number of bacteria (per ml)										
Total bacteria (10^{11})	4.12	1.71	0.65	3.22	3.16	2.37	0.71	0.011	0.571	0.180
F.succinogenes(10^{10})	6.43	4.75	0.63	6.91[a]	6.02[ab]	3.72[b]	0.73	0.090	0.040	0.408
R. albus (10^5)	1.32	0.65	0.37	0.44	1.04	1.44	0.35	0.075	0.163	0.224
R. flavefaciens (10^6)	1.26	0.34	0.44	0.18	0.52	1.71	0.55	0.172	0.106	0.235
VFA (mol/100 mol)										
Acetic acid (C2)	60.8	56.6	1.1	62.9[a]	58.5[ab]	54.8[b]	1.3	0.015	0.003	0.289
Propionic acid (C3)	19.4	21.0	0.9	22.0[a]	21.3[ab]	17.3[b]	1.1	0.251	0.019	0.947
Butyric acid (C4)	14.7	10.7	1.1	12.2	13.3	12.5	1.3	0.019	0.807	0.160
C2:C3 ratio	3.2	2.8	0.1	2.9	2.8	3.2	0.2	0.054	0.190	0.935

[a,b] Values within a row with different superscripts are significantly different ($P \leq 0.05$)

C: Impact of concentrate; O: Effect of oil sources; C x O: Interactive effects of concentrate level and oil sources

Concentrate level and oil supplement did not affect DM, CP and NDF digestibility (Table 4). Diets with low concentrate led to significantly higher grass and rice straw intake than did those having high concentrate. Conversely, ME, total DM and CP intakes remained significantly higher with 1.5% concentrate level in the diet. The intake of concentrate, grass and total CP was not influenced by the various oil sources. However, cattle fed soybean diets had the highest intake of rice straw, ME, total DM and NDF, and no significant differences between non-oil and fish oil diets were found.

Table 4. Feed intake and apparent digestibility of cattle under different concentrate levels and oil supplements.

Parameters	Concentrate levels		SEM	Oil sources			SEM	*p* value		
	0.5%	1.5%		No oil	Soybean Oil	Fish oil		C	O	C x O
Initial LW (kg)	189.2	189.6	3.42	188.3	185.3	194.2	4.10	0.991	0.321	0.706
Final LW (kg)	240.3	258.5	3.77	244.5	251.7	251.9	4.62	0.003	0.443	0.643
Concentrate intake (kg DM/day)	1.07	3.27	0.05	2.14	2.16	2.21	0.06	0.000	0.570	0.990
Grass intake (kg DM/day)	2.13	2.03	0.03	2.06	2.12	2.07	0.04	0.048	0.407	0.004
Rice straw intake (kg DM/day)	1.66	0.94	0.02	1.22^{b}	1.43^{a}	1.25^{b}	0.03	0.000	0.000	0.277
Total DM intake (kg/day)	4.86	6.25	0.07	5.41^{b}	5.72^{a}	5.53^{ab}	0.08	0.000	0.047	0.081
Total CP intake (g/day)	469	778	8.22	611	632	627	9.86	0.000	0.328	0.261
Total NDF intake (kg/day)	2.90	2.89	0.03	2.81^{b}	3.02^{a}	2.85^{b}	0.06	0.902	0.005	0.016
ME intake (MJ/day)	47.7	59.8	0.63	45.1^{b}	57.9^{a}	58.2^{a}	0.76	0.000	0.000	0.571
FCR (kg feed DM/ kg LWG)	8.59	8.18	0.12	8.69^{a}	7.83^{b}	8.64^{a}	0.15	0.033	0.001	0.161
DM digestibility (%)	65.1	63.9	1.65	63.5	66.1	63.7	1.79	0.179	0.081	0.257
CP digestibility (%)	65.2	67.1	1.63	65.8	66.5	65.9	1.77	0.046	0.766	0.434
NDF digestibility (%)	57.7	54.8	1.97	54.1	57.5	57.2	2.19	0.060	0.127	0.011

SEM: Standard error of mean; DM: Dry matter; CP: Crude protein; LW: Live weight; LWG: Live weight gain; FCR: Feed conversion ratio; [a,b] Values within a row with different superscripts are significantly different ($P \leq 0.05$); C: concentrate level; O: oil sources; C x O: concentrate levels x oil sources.

Diets containing 1.5% concentrate and diets with soybean oil gave rise to higher daily weight gain (DWG) of 768 g/day and 738 g/day, respectively (Table 5). The combination of 1.5% concentrate and soybean achieved the highest DWG (851 g/day). Addition of soybean oil to both concentrate diets resulted in the highest DWG of cattle for the entire duration of the experiment.

Table 5. Effects of concentrate levels and oil source supplements on daily weight gain of cattle.

Factors		Daily weight gain (g/day)			
Concentrate levels	Oil sources	0-30 day	31-60 day	61-90 day	Average
Effect of concentrate levels					
0.5%	-	471	554	677	567
1.5%	-	728	836	742	768
SEM		20.7	23.5	16.6	11.9
p value		0.000	0.000	0.012	0.000
Effect of oil sources					
-	No oil	575	675^{ab}	622^{b}	624^{b}
-	Soybean oil	596	760^{a}	858^{a}	738^{a}
-	Fish oil	627	649^{b}	648^{b}	641^{b}
	SEM	25.4	28.8	20.3	14.6
	p value	0.374	0.035	0.000	0.000
Concentrate level x oil source interaction					
0.5%	No oil	415	565	600^{c}	527
0.5%	Soybean oil	509	600	767^{b}	626
0.5%	Fish oil	487	497	662^{bc}	548
1.5%	No oil	735	785	644^{bc}	721
1.5%	Soybean oil	684	920	949^{a}	851
1.5%	Fish oil	767	802	634^{c}	734
SEM		35.9	40.7	28.7	20.7
p value		0.145	0.427	0.006	0.609

[a,b,c] Values within a column with different superscripts are significantly different ($P \leq 0.05$)

DISCUSSION

Rumen parameters: The results indicated that high level of concentrate was associated with low fiber intake, which may affect rumen characteristics, particularly rumen pH. Chenost and Kayouli (1997) reported that cattle fed higher concentrate diet had lower pH in rumen fluid. Melaku *et al.* (2004) found that the activity of fiber-breakdown microorganisms was inhibited with rumen pH below 6.1. In this study, rumen pH was lower at higher level of concentrate but remained above 6.1 (Table 3), hence it did not affect fiber fermentation and the synthesis of microbial protein. The absence of change in the rumen pH with different oil supplements is in line with the findings of Lin *et al.* (2012). Shingfield *et al.* (2008) reported that supplementation with sunflower oil tended to reduce ammonia level whereas fish oil supplementation increased ammonia level (Keady and Mayne, 1999). However, the present study did not show such tendency and instead is in agreement with the results of Li *et al.* (2015) and Parvar *et al.* (2017) that unlike non-supplemented diets, those with linseed oil or soybean oil and fat supplementation did not alter NH_3-N content.

High concentrate level in diet did not support the growth of rumen bacteria (1.71×10^{11}/ml) as compared to low concentrate level (4.12×10^{11}/ml). Generally, concentrate is an easily fermented feed that promotes the growth of rumen bacteria. However, this study demonstrated that high concentrate level (1.5% DM) had an adverse effect on whole bacterial community in rumen, particularly the cellulytic bacteria (Table 3). Similarly, Granja-Salcedo *et al.* (2016) also showed that the number of cellulolytic bacteria including *R. flavefaciens* and *R. albus* was low in the rumen of steers fed high amount of concentrate. In our study, fish oil supplemented to the diet reduced the number of total bacteria and *F. Succinogenes* and increased the quantity of *R. albus* and *R. Flavefaciens*. In line with our results, Yang *et al.* (2009) indicated that supplementation of oil to cattle diets reduced the number of fibrous microorganisms significantly but did not have effect on the total number of bacteria. In addition, Chen and Weimer (2001) observed that the development of *R. flavefaciens* and *F. succinogenes* was inhibited by the presence of *S. ruminantium* and *R. albus*. The competition between microbial species in the rumen may alter the amount of *F. Succinogenes* and this might help explain why *F. succinogenes* was significantly reduced but two groups of *R. albus* and *R. flavefaciens* remained stable with the supplementation of both oil types in the present diets.

Fish oil suplemented diet led to lower content of acetic and propionic acid than non-oil and soybean suplemented diet (Table 3). The findings were similar to those of Li *et al*. (2011) and Shingfield *et al*. (2011), who indicated that fish oil supplemented diet resulted in consistenly lower acetic acid concentration in rumen fluid than did the addition of plant originated oils, such as linseed and safflower oil. A possible explanation could be that the density of *F. succinogenes*, acetate-producing bacteria, on fish oil diet was lower than that of soybean oil and non-oil diets. Acetate to propionate ratio (C2:C3) is directly influenced by the diet composition and with more roughage consumed, the higher ratio of C2:C3 was recorded as expected in the present study. Chen and Weimer (2001) explained a relationship between variation in VFA and microbial population and reported that on high-forage diet, supplementation of concentrate reduced the number of *F. succinogenes*, *R. flavefaciens*, *B. Fibrisolvens* and *R. albus,* leading to a decrease in VFA production. Consistent with this view, the current study also indicated that the supplemention of fish oil decreased the amount of *F. succinogenes* as well as acetic acid content, compared to the non-supplemented diets. In consensus with Maia *et al.* (2007) and Zhang *et al.* (2008), it can be inferred that acetate-producing bacteria, such as *F. succinogenes*, may be inhibited by polyunsaturated fatty acids.

Feed intake and weight gain: Cattle receiving high level of concentrate in the diet exhibited high live weight gain (Table 4 and 5) and apparently higher intake of ME and CP. The cattle fed on soybean oil supplemented diet gained more weight compared to those receiving diets with non-oil and fish oil. Generally, oil in diet provides the energy needed for the growth of the bacterial community in the rumen (Seng *et al.*, 2001). Soybean oil is known as the main source of unsaturated fatty acids and when added to diets is converted to stearic acid (Harfoot and Hazlewood, 1997) which significantly increases the body weight of cattle (Kita *et al.*, 2003). Unsaturated fats are biohydrogenated by rumen bacteria, after which they are converted to linoleic acid, oleic acid, and linolenic acid into stearic acid (Van de Vossenberg and Joblin, 2003). Stearic acid supplementation was demonstrated to be effective in increasing cattle weight gain (Kita *et al.*, 2003). Fish oil has low content of unsaturated fatty acids and may result in low concentration of stearic acid in the rumen. Cattle fed on fish oil supplement had lower number of rumen bacteria and lower concentration of end products (acetic and propionic acid) as compared to those having soybean oil supplement (Table 3), indicating lower microbial fermentation on diets containing fish oil. Furthermore, soybean oil treatment resulted in significantly greater intake of rice straw, total DM and NDF and ME than did fish oil and non-oil treatments. Another point worth mentioning is that cattle fed soybean oil had lower FCR value than those having fish oil and non-oil diets. Although there was no significant interaction of concentrate level and oil supplementation for weight gain of the cattle (Table 4), supplementation of both concentrate and soybean oil had positive effect on weight gain (Table 5). The results clearly showed that DWG of cattle feeding on soybean supplemented diet significantly increased with 1.5% concentratre level in the diet. Moreover, at both levels of concentreate tested, cattle supplemented with soybean oil had the highest average DWG.

Conclusion: Different concentrate levels and oil sources had significant effect on rumen characteristics and growth of cattle. Cattle fed high concentrate level (1.5%) had lower number of total bacteria in the rumen, higher intake of total DM, total CP and ME, and higher weight gain. Cattle supplemented with soybean oil had higher rumen bacterial density, consumed more feed and had lower FCR and more importantly, achieved higher weight gain as compared to those fed fish oil and non-oil diets. The supplementation of concentrate and fish oil had additive effect on weight gain of the cattle. It can be suggested that the inclusion of concentrate and/or soybean oil in the diet of growing cattle help achieve higher weight gain.

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