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In Vitro Assessment of the Defaunating Capacity of Various Types and Levels of Supplemental Lipid on Rumen Liquor of Goat (*Capra hircus* Linn.)

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ABSTRACT

Effects of supplemental lipid on rumen microbial population vary because of their differences in fatty acid profiles. Hence, preliminary assessment of lipid sources and knowing the right amount to add up in the diet is a key for lipid supplementation to be successful. This study was designed to investigate the defaunating capacity of various types of lipids added to rumen liquor *in vitro* at 3% and 6% dry matter intake per day (DMI/d) of goat in relation to rumen volume and body weight. The experiment was set up in a Completely Randomized Design (CRD) with seven treatments: T0 – without fat, T1 – Corn oil at 3%, T2 - Corn oil at 6%, T3 – Coconut oil at 3%, T4 - Coconut oil 6%, T5 – Lard fat at 3% , T6 - Lard fat at 6%, replicated three times. Rumen liquor was collected from goat weighing fifteen kilograms via oral stomach tubing connected to syringe, thereafter, microbial population was evaluated before and after lipid addition. Results revealed that corn oil, having highest percentage of long-chain polyunsaturated fatty acids showed the greatest defaunating capacity for protozoa in comparison to coconut oil and lard fat containing more medium chain saturated fatty acids and long-chain monounsaturated fatty acids, respectively. It was also found that corn oil caused the lowest reduction in bacterial population and minimal fluctuation in rumen liquor pH. It is, therefore, recommended to use corn oil up to 6% of DMI/d, but coconut oil and lard fat be added up to only 3% DMI/d.

Keywords: *In vitro*, rumen liquid, lipids, defaunation, goat

INTRODUCTION

Most rural farmers nowadays still raise small ruminant animals such as goat (*Capra hircus*) in traditional way while others in larger scale of production, have already adopted the feedlot operation under cut - and - carry method of feeding. [1,2] In ruminant nutrition, it is a common practice to partially replace grain with fats to avoid metabolic stresses associated with low fiber diets, like ruminal acidosis. [3] Aside from that, added fat particularly unsaturated fat, also confers benefit to the animal by means of defaunating the rumen resulting in decreased protozoal counts, [4] increase in bacterial growth efficiency,[5] and improvement in body weight gain and feed conversion efficiency. [6]Since bacteria are engulfed by the protozoa, [7] the reason for this seems to be based on the availability of an ecological niche for the bacteria when protozoa are absent in the rumen combined with the removal of protozoal predation, [8] suppression of protozoa in the rumen might therefore improve and optimize bacterial activity and finally increase the efficiency of bacteria for dietary fiber digestion.

Economically, fat is incorporated in the animal diet because it is cheaper to some extent and contains 2.25 times energy than carbohydrates, [9] so that small addition can drastically increase the energy content of the diet. However, one of the issues behind fat supplementation in ruminants is the biohydrogenation process that occurs inside the rumen which increase the content of

saturated fatty acids in the meat that consequently increase the risk of cardiovascular diseases. [10] Microbial biohydrogenation is a unique process prevalent in the rumen that converts unsaturated fatty acids to more saturated end products which result in deposition of body fat in a saturated form. The addition of fat, however, becomes beneficial when the rumen turns out to be defaunated and bacterial population is consequently increased, for instance to 1.14 x 10^2 colonies/ml and fiber digestibility to 59.4%. [11]

Defaunation has been a subject of debate due to inconsistent findings, [12] wherein some research results indicate that defaunation enhances animals feed intake, feed digestion, rumen metabolism and weight gain while some research results showed reverse effects of defaunation by impeding this parameters. Several researchers studied several kinds of treatments to achieve partial or complete defaunation in the rumen which include chemicals that are toxic to protozoa (copper sulfate, dioctyl sodium sulfosuccinate, alcohol ethoxy-late or alkanates, calcium peroxide), ionophores, lipids, and saponins. [8,13,14] They pointed out that among the treatments, lipids were predominantly used for protozoal control as well as in methane mitigation approach. However, limited experiment has been conducted comparing the effects of lipids on rumen microbial population *in vitro*. Therefore, this *in vitro* experiment was conducted to find out the defaunating capacity of different types of lipids such as corn oil, coconut oil and lard fat at different levels of addition on rumen microbial population of goats.

MATERIALS AND METHODS

Preparation of Treatments

Commercially produced corn oil and coconut oil were purchased and used in this experiment. On the other hand, lard (hog fat) from the tissue covering the belly (belly trimmings) of the pig was utilized as source of fat for this study. Belly trimmings were purchased from the wet market of Baybay City, Leyte, Philippines. It was chopped into 2-3 inches long and heated in an open flame until it melts. The melted fat was strained with cheesecloth, placed inside a glass container, and stored in room temperature (Figure 1). The selection of fats for this experiment was based on lipids' saturated and unsaturated fat content considering their varying fatty acids component (Table 1.)

Table 1. Fatty acid composition of added lipids to rumen liquor *in vitro*. [15]

Corn oil	16% Saturated	84% Unsaturated
	13% Palmitic (16:0)	52% Linoliec (18:2 n-6)
	3% Stearic (18:0)	31% Oleic (18:1 n-9)
		1% Linolenic (18:3 n-3)
Coconut Oil	90% Saturated	9% Unsaturated
	48% Lauric (12:0)	7% Oleic (18:1 n-9)
	16% Myristic (14:0)	2% Linoleic (18:2 n-6)
	9% Palmitic (16:0)	
	8% Caprylic (18:0)	
	7% Capric (10:0)	
	2% Stearic (18:0)	
		Others 1%
Lard (hog fat)	40% Saturated	Unsaturated 59%
	27% palmitic (16:0)	44% Oleic (18:1 n-9)
	11% Stearic (18:0)	11% Linoleic (18:2 n-6)
	2% Myristic (14:0)	4% Palmitoleic (16:1 n-7)
		0thers 1%

Treatments and Experimental Design

The 3% and 6% lipid were supplemented to the rumen liquor based on average dry matter intake (DMI) of goats per day in relation to rumen volume and body weight. This *in vitro* experiment had the following treatments:

T_0 –without fat
T_1 - Corn oil at 3% of DMI/d
T_2 - Corn oil at 6% of DMI/d
T_3 – Coconut oil at 3% of DMI/d
T_4 - Coconut oil at 6% of DMI/d
T_5 - Lard fat at 3% of DMI/d
T_6 - Lard fat at 6% of DMI/d

This combination of treatments was set – up in a Completely Randomized Design (CRD) at the College of Veterinary Medicine Laboratory, Visayas State University, Baybay City Leyte, Philippines. The rumen liquor used in this experiment came from one weanling goat aging 6 months old with fifteen (15) kilograms live weight.

Sampling Procedures

Goat in this study were treated properly in accordance with the Provisions of Republic Act 8485 or the Animal Welfare Act of 1998. It was fed daily with a diet consisting of Napier grass (*Pennisetum purpureum*) at 3% of its body weight in a dry matter (DM) basis. Rumen liquor samples were aspirated from the rumen by inserting orally the rubberized stomach tube (CH/FR 18) connected to a 20 ml plastic syringe. [16] After collection, it was filtered with double layer cheesecloth to separate the feed particles from the rumen sample. Then, pH of rumen liquor was immediately measured through Milwaukee SM101 digital pH meter with glass electrode and transferred into a pre-heated glass vial ensuring anaerobic condition by lighting a flame inside the vial to remove oxygen. Samples of rumen liquor were brought to the laboratory for protozoal and bacterial counting.

Culture Condition

Rumen liquor was used as the substrate/ culture medium *in vitro* to determine the effect of different types and levels of lipid on rumen bacterial and protozoal population. Culture condition was set – up by pipetting 10ml of rumen liquor fluid from the glass vial and transferred into the test tube. This test tube was added with different types of fat using 1ml pipette at 3% (8.9mg) and 6% (17.7mg) of the required dry matter intake (DMI) of goats according to its body weight in relation to rumen volume (4 gallons). Test tubes containing the test samples were placed into a hot water shaker bath for 48 hours to constantly mix the lipid on rumen liquor and allow action of microorganism on the substrate, [17] thus simulate rumen motility. Temperature was set at 41ºC to mimic rumen environment because after the animal eats, temperature rises to this level due to the fermentation process that generates heat. [18]

The amount of lipid added at 3% and 6% were calculated as follows:

Assumptions:

1. Goats weighing 15kg will consume Dry Matter (DM) at 3% of body weight.
2. Rumen volume is approximately 4 gallons. 1 gallon is equivalent to 3.8 liters.
   4 gallons x 3.8 L = 15.2L

Calculation for lipid supplemented to the substrate.

15 kg BW x 3% = 450gm DMI/d

➢ **3%** lipid	**6%** lipid
450gm DMI/d x 3% = 13.5gm fat	450gm DMI/d x 6% = 27gm fat
$\frac{13.5\text{gm fat}}{15.2\text{L}}$	$\frac{27\text{gm fat}}{15.2\text{L}}$
$= \frac{0.89\text{gm}}{1000\text{ml}}$	$= \frac{1.77\text{gm}}{1000\text{ml}}$
= 0.00089gm x 10ml	= 0.00177 x 10ml
= 0.0089gm/10ml	= 0.017gm/10ml
$= \frac{0.0089\text{gm x } 1000\text{mg}}{1\text{gm}}$	$= \frac{0.017\text{gm x } 1000\text{mg}}{1\text{gm}}$
=8.9mg fat/ 10 ml rumen liquor	= 17.7mg fat/10ml rumen liquor

Bacterial Counting

Serial dilution and plate count method were performed to determine the bacterial population. [19] After 48 hours of incubation, a subsample of 1ml was taken from the test tube using a 5ml (3mm) wide pipette and serially diluted into 1:1000, 1:10,000, and 1:100,000 and 1:1,000,000 for bacterial count. The standard plate count (SPC) per ml of sample was determined using pour plate technique. It was done by pipetting 1ml sample of every dilution into sterile petri plates. Each petri plate on the other hand contained 25ml of liquefied Plate

Count Agar (PCA) growth medium heated under temperature of 41°C. Thereafter, sample and melted agar were mixed carefully into the prepared petri plates and was incubated under anaerobic condition at 40°C using GasPak anaerobe container sachet for 24 hours. Each treatment replicates were analyzed in duplicate. Any bacterial colonies formed after 24 hours were counted using "Suntex" colony counter. Colony forming units (cfu)/mL were computed based on this formula: cfu/mL = number of bacterial colonies x dilution rate.

Protozoal Count (Subsampling, Staining and Dilution)

The number of protozoa was counted based on the modified using a Sedgewick Rafter counting chamber. [20] A 1ml of treated rumen liquor sample was poured using a 5ml pipette with a wide orifice (3 mm) into the test tube. Three drops of lugols solution were added into the test tube and allowed to stand for at least 15 minutes.

Afterwards, 9ml of 30% glycerol solution was added into the test tube resulted in a 1:10 dilution of the original rumen contents. The 30% glycerol solution was used because it has high – enough viscosity to prevent rapid settling of protozoa during the process of pipetting subsamples for counting. 1ml of diluted sample was pipetted into a Sedgewick rafter counting chamber by a wide-orifice pipette and examined under optical Olympus (CX11) microscope.

The number of protozoa was counted at a 10x magnification. All grids evenly spaced over the entire chamber surface were counted. Multiplying the total number of protozoa counted to the dilution factor which is 10 gives the total number of protozoa per milliliter of diluted rumen liquor. Protozoal concentration was expressed on a logarithmic basis (log [no. cells/ml]).

Data Gathered

1. pH of Rumen Liquor

The pH of rumen liquor was measured using Milwaukee SM101 digital pH meter with glass electrode before and after incubation period of 48 hours.

2. Bacterial Count (cfu/ml)

It was computed using this formula:

cfu/mL = number of bacterial colonies x dilution rate.

3. Protozoal Count

Protozoal population per ml of rumen liquor was computed using this formula:

Total number of protozoa counted x Dilution factor

Analysis of Data

The obtained data were subjected to one - way analysis of variance (ANOVA) and comparison of means was carried out by Tukey's Honestly Significant Difference (HSD) Test using Statistical Package for Social Science (SPSS) version 20.

RESULTS AND DISCUSSIONS

Protozoal population dynamics *in vitro*

The fatty acid profiles used in this study are displayed in Table 1 while summarized in Table 2 are the changes in protozoal population in the rumen liquor as affected by different types and levels of lipid added at 3% and 6% DMI/d in relation to rumen volume *in vitro*. It appears that all types of lipid added at different levels are toxic to rumen protozoa (P<.001). However, the greatest defaunating effect was observed in corn oil (T_1, T_2) even up to 6% addition. This is followed by lard fat (T_5) and coconut oil (T_3), respectively. On the other hand, lowest reduction in protozoal numbers was noticed with lard fat (T_6) and coconut oil (T_4) at 6% addition. In this case, protozoa responded and tended to be sensitive in corn oil even up to 6% (T_2) addition compared to other treatments. This indicates that fat with more percentage of long - chain polyunsaturated fatty acids is more toxic to rumen protozoa than those fats with more monounsaturated fatty acids such as lard fat and medium chain saturated fatty acids such as coconut oil. This finding confirms the result of other researchers using live animals

(*in vivo*) that addition of corn oil to diet reduced rumen protozoal population. [11]

The attempts to maximize animal production lead various researchers to defaunate the rumen using lipid that came from different sources. [21,22] According to Moussavi *et al*., (2008), [23] regardless of degree of saturation, the fatty acids are incorporated into the cell membranes of organisms. From this hypothesis, it could be that the antimicrobial property of the highest percentage of fatty acids comprising the type of fat such as linoleic acid in corn oil, [24] lauric acid in coconut oil, [25] and oleic acid in lard fat, [26] triggered the death and eventually reduction of protozoal population in rumen liquor. In addition, it was reported that higher number of unsaturated bonds present in additional fat is responsible for decreasing trend in protozoal counts. [4,17]

Table 2. Protozoal population of rumen liquor *in vitro* as influenced by added lipids at 3% and 6% of average dry matter intake (DMI) of goats per day in relation to rumen volume and body weight

Treatments	Protozoal population ($x10^4$/ml of rumen liquor)		% change in protozoal population
	Pre - treatment	Post - treatment	
T_0	53.9	53.9^{a}	0
T_1	53.9	3.3^{c}	-93.63^{c}
T_2	53.9	1.5^{c}	-97.13^{c}
T_3	53.9	18.4^{b}	-65.68^{ab}
T_4	53.9	24.2^{b}	-54.41^{ab}
T_5	53.9	12.2^{bc}	-77.61^{bc}
T_6	53.9	30.4^{b}	-42.72^{a}
p - value		0.001**	0.001**

** Highly Significant (P<0.001). Treatment means within a column having the same letter superscripts are not significantly different from each other. Treatments: T0 - Without fat; T1 – Corn oil at 3% DMI/d; T2 – Corn oil at 6% DMI/d; T3 – Coconut oil at 3% DMI/d; T4 – Coconut oil at 6% DMI/d; T5 – Lard fat at 3% DMI/d; T6 – Lard fat at 6% DMI/d

Bacterial population dynamics *in vitro*

Table 3 show the effects of different types and levels of lipid on bacterial population of rumen liquor *in vitro* Treatments effects were analyzed in comparison with the control group (T_0, without fat addition). Results showed that bacterial population was significantly (P<.001) affected by different types and levels of lipid addition *in vitro*. It can be observed that T_1, T_2 and T_3 groups had slight reduction in bacterial population comparable to control group (T_0), whereas T_4 and T_5 and T_6 had the highest reduction. It can be observed that as the level of corn oil and coconut oil increases bacterial population slightly decreases, but not with saturated lard fat. As such, addition of corn oil containing more of polyunsaturated fatty acids up to 6% (DMI/d) is ideal since it decreases the population of rumen protozoa (Table 2) while having minimal reduction in rumen bacteria.

The metabolic fate of fatty acids component of fat is determined by its structural characteristics including carbon chain length (short, medium, long) and number of double bonds or degree of saturation (saturated, monounsaturated, or polyunsaturated). Generally, it may be oxidized for energy, incorporated into cell membranes, utilized for synthesis of biologically active compounds, or deposited into adipose tissue to provide an energy source. [27] The mechanism behind the reduction of bacterial population by different types of fat could be possibly due to penetration or integration of their dominating fatty acid component with antimicrobial activity such as the linoleic acid, [24] lauric acid, [25, 28] and oleic acid [26] that leads to the disruption of their lipid membrane and cell. This study, to some extent, supports the findings of Zain *et al*., (2008) that feeding of corn oil and coconut oil respectively promote positive microbial balance in favor to bacteria inside the rumen. [11]

Table 3. Bacterial population of rumen liquor *in vitro* as influenced by added lipids at 3% and 6% of average dry matter intake (DMI) of goats per day in relation to rumen volume and body weight.

Treatments	Bacterial Population ($x10^7$ cfu/ml)		% Change in bacterial population
	Pre - treatment	Post - treatment	
T_0	23.4	23.4^{a}	0
T_1	23.4	22.2^{a}	-3.26^{a}
T_2	23.4	21.2^{a}	-9.31^{a}
T_3	23.4	21.8^{a}	-7.70^{a}
T_4	23.4	7.5^{b}	-67.50^{b}
T_5	23.4	6.8^{b}	-71.44^{b}
T_6	23.4	8.7 ^{b}	-61.85^{b}
p - value		0.001**	0.001**

** Highly Significant (P<0.001). Treatment means within a column having the same letter superscripts are not significantly different from each other. Treatments: T0 - Without fat; T1 – Corn oil at 3% DMI/d; T2 – Corn oil at 6% DMI/d; T3 – Coconut oil at 3% DMI/d; T4 – Coconut oil at 6% DMI/d; T5 – Lard fat at 3% DMI/d; T6 – Lard fat at 6% DMI/d

Changes in pH

After 48 hours of incubation, changes in rumen liquor pH was noted (Table 4). All types and levels of fat added significantly influenced (P<0.001) the pH of rumen liquor. It can be observed that increasing the level of fat addition from 3% to 6% DMI/d into rumen liquor *in vitro* is accompanied with a slight increase in acidity. Although results are statistically comparable, it appears that addition of corn oil at 3% (T_1) and 6% (T_2) obtained the minimal, whereas coconut oil and lard fat at 3% (T_3, T_5) and 6% (T_4, T_6) group had the highest reduction in rumen liquor pH compared to the control group (T_0). Although changes in rumen liquor pH as affected by addition of different types and levels of fat were observable, such reductions were very minimal, maintaining the pH within normal range of 5.5 to 7.0. [29]

The slight increase in acidity of rumen liquor exhibited by all treatments might be the result of free fatty acid liberated from the lipid, which can be linked to toxic effect of fat in rumen protozoa. [30] It was hypothesized that when the bacterial and protozoal cell is exposed under acidic environment their intracellular pH gradually decreases and this triggers the inhibition of their growth. A study of Machmuller *et al*., (2003) indicated that defaunation reduces rumen pH, however, it is accompanied with increase in bacterial concentration. [31]

Table 4. pH of rumen liquor *in vitro* as influenced by added lipids at 3% and 6% of average dry matter intake (DMI) of goats per day in relation to rumen volume and body weight.

Treatments	Pre - treatment	Post treatment	%change in pH
T_0	7.02	6.89^{a}	-1.89^{a}
T_1	7.02	6.74^{ab}	-3.94^{ab}
T_2	7.02	6.37^{abc}	-9.21^{abc}
T_3	7.02	6.33^{bc}	-9.82^{bc}
T_4	7.02	5.98^{c}	-14.81^{c}
T_5	7.02	6.47^{abc}	-7.78^{abc}
T_6	7.02	6.28^{bc}	-10.58^{bc}
p – value		0.001**	0.001**

** Highly Significant (P<0.001). Treatment means within a column having the same letter superscripts are not significantly different from each other. Treatments: T0 - Without fat; T1 – Corn oil at 3% DMI/d; T2 – Corn oil at 6% DMI/d; T3 – Coconut oil at 3% DMI/d; T4 – Coconut oil at 6% DMI/d; T5 – Lard fat at 3% DMI/d; T6 – Lard fat at 6% DMI/d

CONCLUSIONS

Different types of lipid added at different levels numerically reduce bacterial and protozoal population of rumen liquor *in vitro*. As the level of lipid addition in the rumen liquor increases (6%, DMI/d), reduction in bacterial population also increases together with the pH. Such negative effect, however, is outweighed by drastic decrease in protozoal numbers which may confer positive benefits on animal performance. Considering their variable fatty acid content, corn oil can potentially be used as defaunating agent up to 6% level of the daily dry matter intake (DMI) since it demonstrates reduction on protozoal numbers with minimal reduction in bacterial population. However, when using coconut oil and lard fat it is better to limit the addition at 3% of the DMI/d.

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