Journal of Environmental Treatment Techniques 2019, Special Issue on Environment, Management and Economy, Pages: 1146-1156

J. Environ. Treat. Tech.
ISSN: 2309-1185

Journal web link: http://www.jett.dormaj.com

Association of Rumen Fluid Analyses and Nutrient Apparent Digestibility in Goats as Influence by Fat Supplementation

Niel L. Ningal[1], Nora C. Cabaral[2]*

[1]University of the Philippines, Los Bańos, College of Agriculture and Food Science, Los Bańos, Laguna, Philippines
[2]Mindoro State College of Agriculture and Technology, Department of Animal Science, Oriental, Mindoro, Philippines

Received: 13/09/2019 Accepted: 22/11/2019 Published: 20/12/2019

Abstract

Goat nutrition depends on rumen microorganisms, however when rumen ecosystem is disturbed, cascade of detrimental effects on animal health and productivity takes place. Thus, rumen ecosystem alteration by supplementing dietary fats with known potential in microbial protein synthesis, nutrient digestibility and reduction of ruminal NH3-N emission are indeed significant scientific challenge. The study aims to explore the potential of dietary fats supplementation in goats. Three rumen-cannulated goats in individual metabolism stalls with customized urine collection tools was used following cross-over trial over time. Animals were randomly selected on different dietary treatment and cycle, provided with concentrate supplemented with VCO and lard at 3 and 5% on the morning and ad libitum feeding of Napier grass thereafter with continuous access to clean drinking water. Result showed that there were positively high associations in all nutrients (DM, CP, NDF and ADF) apparent digestibility while negatively high association between rumen pH and NH3-N. Moderately positive associations were observed between purine derivatives and apparent nutrient digestibility. However, moderately negative relationship between VFA and purine derivatives and between NH3-N and rumen temperature. In general, inversely proportional association on rumen pH and NH3-N was noted. However, apparent CP, NDF and ADF digestibility has strong positive association.

Keywords: Rumen digestibility, Microbial protein synthesis, NH3-N emission, VCO, Lard

1 Introduction

It is of paramount importance that the animal be fed appropriately so that the ruminal organisms stay healthy. Since the health and productivity of the goat depends on the rumen function, without these microorganisms the animal will become very sick and eventually died. Thus rumen organisms require fiber, nitrogen (protein), and energy (carbohydrates). Roughages supply fiber, whey provides protein, and energy is provided by good quality (digestible) roughages and by concentrates (grains). The rumen microorganisms that digest fiber thrive but in a pH range of 6.0-6.8 (3). The vigorousness with which the microbes break down food entering the rumen would be based upon the rate at which they are growing and reproducing. Sufficient energy to meet their requirements is needed for them to grow rapidly. Good sources of "instant energy" are nutrient source that usually contains high proportion of α-linked polysaccharides which can be in the form of grain such as maize or sorghum or a sugar by-product such as molasses. Moreover, adding nitrogen supply has similar effects to adding energy because a source of nitrogen is needed by the microbes so as to make their own body proteins (2).

Common nitrogen source of nitrogen in ruminant diet is urea which is classified as a non-protein nitrogen (NPN) supplement. Urea has 46% N which is equivalent to 28% crude protein, but it could be toxic if given in higher amounts. Since the ammonia formed from urea is toxic at high concentrations in the body, it should not be used at more than 1% of the total dry matter intake of the animal (16). The rumen ammonia nitrogen comes from the deamination of dietary proteins. This also comes from non-protein nitrogen (NPN) and from urea derived both from the saliva and from the blood after diffusion across the fore stomach wall. High levels of intraruminal ammonia are produced once ruminants are fed excess protein or urea supplements. This could lead to energy waste in urea synthesis and a potential to ammonia toxicity. Moreover, Stern and Bach mentioned that ruminant nutrition depends on rumen microorganisms that allow the animals to harness the potential of low energy feedstuffs (18). Without rumen microorganisms, or when the rumen ecosystem is disturbed due to different unfavorable

Corresponding author: Niel L. Ningal, University of the Philippines, Los Bańos, College of Agriculture and Food Science, Los Bańos, Laguna, Philippines. Email: nielningal.qualipeak@gmail.com.

environmental factors (i.e. high temperature, low plane of nutrition), there can be a cascade of detrimental effects on animal health and productivity. One way to alter the rumen ecosystem is by supplementing dietary fats that is known to influence the synthesis of microbial protein, ruminal ammonia nitrogen emission, rumen temperature and pH, and eventually facilitates the digestibility of different nutrients. Hence, this study was conducted.

In addition, researchers reported that when oil is included in the feed concentrate, it converts unsaturated fats to the saturated state by acquiring the hydrogen in the rumen environment competing with the potential methane produced. Thus, lessening the carbon emissions and has now provided new sources to nourish the animal by the saturated fats. It is aimed to find ways not only to reduce the methane emissions of ruminants but also to help improve the animals' nutrition and productivity (4-13) Hence, this study was conducted to determine the association of rumen fluid analyses and nutrient apparent digestibility in goats as influence with various dietary fat supplementations.

The results may serve as basis for feed companies, government and private commercial goat raisers and guide researchers and goat experts in identifying feeding and nutrition aspects that need further improvement. This study serves as a springboard for identifying S&T interventions necessary to enhance goat farm productivity while reducing GHGs emission and provide decision support not only for ruminant nutritionist and goat raisers but also for extensionists, researchers and policy makers.

2 Materials and Methods

The study was conducted from August 4, 2015 to April 22, 2016 at the Metabolism Laboratory of the Institute of Animal and Dairy Sciences Cluster (ADSC), and Biotechnology Laboratory at the University of the Philippines, Los Baňos, College, Laguna.

Three (3) female (rumen-cannulated goats) weighting 27.33±1.53 kg were housed in individual elevated metabolism stalls provided with 30% concentrate in the morning based on feed requirements (3% of their body weight (BW) dry matter (DM) basis) of the animals. *Ad libitum* feeding of napier grass follows thereafter. Clean drinking water were made available all the times in the respective animal watering troughs. Rumen fluid samples of different treatments were subjected to total volatile fatty acid (TVFAs) production, rumen NH_3-N production, rumen pH, temperature and urine volume for estimation of purine derivatives analysis. Likewise, associations of total VFAs, NH_3-N, purine derivatives estimates, DM, CP and NDF digestibility with the rest of rumen condition and digestibility parameters were tabulated.

2.1 Laboratory Analysis

Evaluation of in situ rumen fluid analyses in rumen-cannulated goats fed with different dietary fats on the concentrate were done through different biochemical analysis of different parameters gathered, rumen pH, temperature, TVAs, NH_3-N and purine derivatives estimates.

2.2. Rumen pH

The pH of the rumen fluid was determined immediately upon collection using a Jenway 3505® portable glass electrode pH meter (Keison Products, United Kingdom).

2.3 Ruminal Temperature, ºC

Rumen temperature of each animal was obtained and recorded using a digital thermometer.

2.4 Total Volatile fatty Acids (TVFA's)

The rumen fluid samples used steam distillation with calculation.

VFA (mmol/100ml) = (titrate (ml) x Na OH factor* x 100)/vol. of rumen fluid.

*NOTE: * 0.05N solution factor should be determined using oxalic acid: Take 1.25ml of saturated NaOH was measured and topped up to 500ml with H_2O. Dissolve using phenolphthalein indicator. The color changes from clear to red.*

2.5 Production Estimation by Purine Derivatives Analyses

To determine the effects of dietary fat on protein nutrition of goats, microbial protein production was estimated by analyzing allantoin in the urine (1). On the average, allatoin and uric acid represent 85% and 15% of the total purine derivative excretion in goats respectively. Urine volume was weighed for the last three day of every 11th day of the in situ trial for each of the treatment cycle. Freshly collected urine samples were diluted to 10:1 with 10% H_2SO_4 to prevent bacterial breakdown of purine derivatives. Urine samples from each animal were further diluted 20X with tap water. Representative samples were kept at 4°C until use.

The method of measuring allantoin was based on the colorimetric method described by Young and Conway (1942). In this procedure, allantoin is initially hydrolyzed under a weak alkaline condition at 100°C to produce allantoin acid which is further degraded to urea and glyoxylic acid in weak acid solution. The glyoxylic acid is then reacted with phynylhydrazine hydrochloride to produce a phenyldrazone of the acid. The product then forms an unstable chromophore with potassium ferricyanide which is read at 522 nm.

Using 15 millimeters (ml) tubes, 1 ml diluted urine sample previously collected and stored at 4°C was thawed at room temperature. The sample was further diluted with 5ml distilled water and 1 ml of 0.5 M NaOH, then incubated in boiling water bath for 7 min. After cooling in cold water bath, 1 ml of 0.5 M HCl and 1 ml of 0.023 M phenylhydrazine solution were added to each test tube and re-incubated in boiling water bath for another 7 min. After cooling in icy ethanol, 3 ml of cooled (-20°C) 11.4 N concentrated HCl and 1 ml of 0.05 M potassium ferricyanide were quickly added to the samples. After mixing, the solution was allowed to stand for 20 min. Absorbance at 522 nm was determined using the Shimadzu model of UV-VIS spectrophotometer (Shimadzu). Concentration of allantoin from urine samples was estimated from a calibration curve using allantoin (Sigma-Aldrich) as standard.

Total urine excretion of allantonic acid was calculated by considering in the calculation the dilution factors and by multiplying the allantoin concentration to the average daily urine excretion of the animal. Computed values were converted as excretion considering that 85% of the total purine derivatives were excreted in the urine of cattle and water buffaloes in the form of allantoin (1). Total purine derivative excretion values were subjected to ANOVA for Crossover Design.

2.6 Rumen Ammonia-N Analysis

Ammonia nitrogen (NH_3-N) concentration was measured using ammonia electrode (Model 95-12) in conjunction with an Orion Ion Analyzer (Model 501 pH/mV meter).

2.7 Research Design and Lay-out

Three mature goats surgically fitted with rumen cannula were used. The experimental animals were in good body condition prior to and throughout the duration of the study. Complete Randomized Design (CRD) was used to evaluate the effect of different dietary treatments. Five dietary treatments were used in the study with dietary treatment combinations as follows.

2.8 Treatment Combinations

The rumen-cannulated goats were supplemented with different levels of two dietary fat sources with dietary treatment combinations as follows.

Treatment 1	- Control
Treatment 2	- 3% Virgin Coconut Oil (VCO)
Treatment 3	- 5% Virgin Coconut Oil (VCO)
Treatment 4	- 3% Lard
Treatment 5	- 5% Lard

Table 1: Treatment assignment of goats for the entire duration of the study

Treatment	Initial	1st Cycle	2nd Cycle	3rd Cycle	4th Cycle
Animal 1	T3	T2	T5	T4	T1
Animal 2	T2	T3	T4	T1	T5
Animal 3	T5	T4	T3	T1	T2

2.9 Statistical Analysis

The statistical analysis was carried out using Statistical Analysis System (SAS® for windows© v.9.1.3. sp.4) software (17). The result of significant at $P<0.05$) were presented. The relationship of rumen condition (temperature, pH, total volatile fatty acids, NH3-N, and purine derivatives) data and nutrient apparent digestibility (dry matter, crude protein, neutral detergent fiber and acid detergent fiber) were collected and tabulated using the following.

$$Yi= a + bx + e$$

Where: a = intercept, b = slope, e= error, x = dependent variable; Y = another dependent variable

3 Result and Discussion

The following relationship of rumen condition (VFAs, RT, pH, PD, NH3-N) and apparent nutrient digestibility (MC, CP, NDF, ADF) of goat supplemented with different dietary fats.

3.1 Correlation Values of Volatile Fatty Acids with other Parameters on Rumen Condition and Apparent Digestibility

Simple and complex carbohydrates (fiber) are digested by rumen microbes and converted into volatile fatty acids. The volatile fatty acids, which consist mainly of acetic, propionic, and butyric acids, are the primary energy source for ruminants. Volatile fatty acid production can be influenced by different factors that could affect the total ruminant production.

The relationship of volatile fatty acids on different rumen condition parameters and apparent nutrient digestibility (DM, CP, NDF, and ADF) as presented in Figure 1. Data showed that VFA have moderately negative relationship on rumen temperature, NH3-N and purine derivatives. On the other hand, negatively low relationship was observed on rumen pH and all apparent nutrient digestibilities (DM, CP, NDF and ADF).

3.2 Correlation Values of Rumen Ammonia Nitrogen with other Parameters on Rumen Condition and Apparent Digestibility

Ruminal ammonia-N concentration often serves as an indicator of N-status for microbial production. Roffler and Satter (14, 15) have presented an equation to predict ruminal ammonia from intake protien and dietary energy density. Ammonia concentration represents the residual balance between input and extraction from the ammonia pool in the rumen.

The relationship of rumen ammonia nitrogen on different rumen condition parameters and apparent nutrient digestibility (DM, CP, NDF, and ADF) as presented in Figure 2. Data showed that NH3-N have positively low association with apparent nutrient digestibility (DM, CP, NDF and ADF). However, very low to moderately negative relationship were observed on temperature, VFA and purine derivatives. While, negatively high relationship was observed in rumen pH. The result suggests that there were decrease of 52% in rumen pH upon increase of 1.0 mg/l of NH3-N.

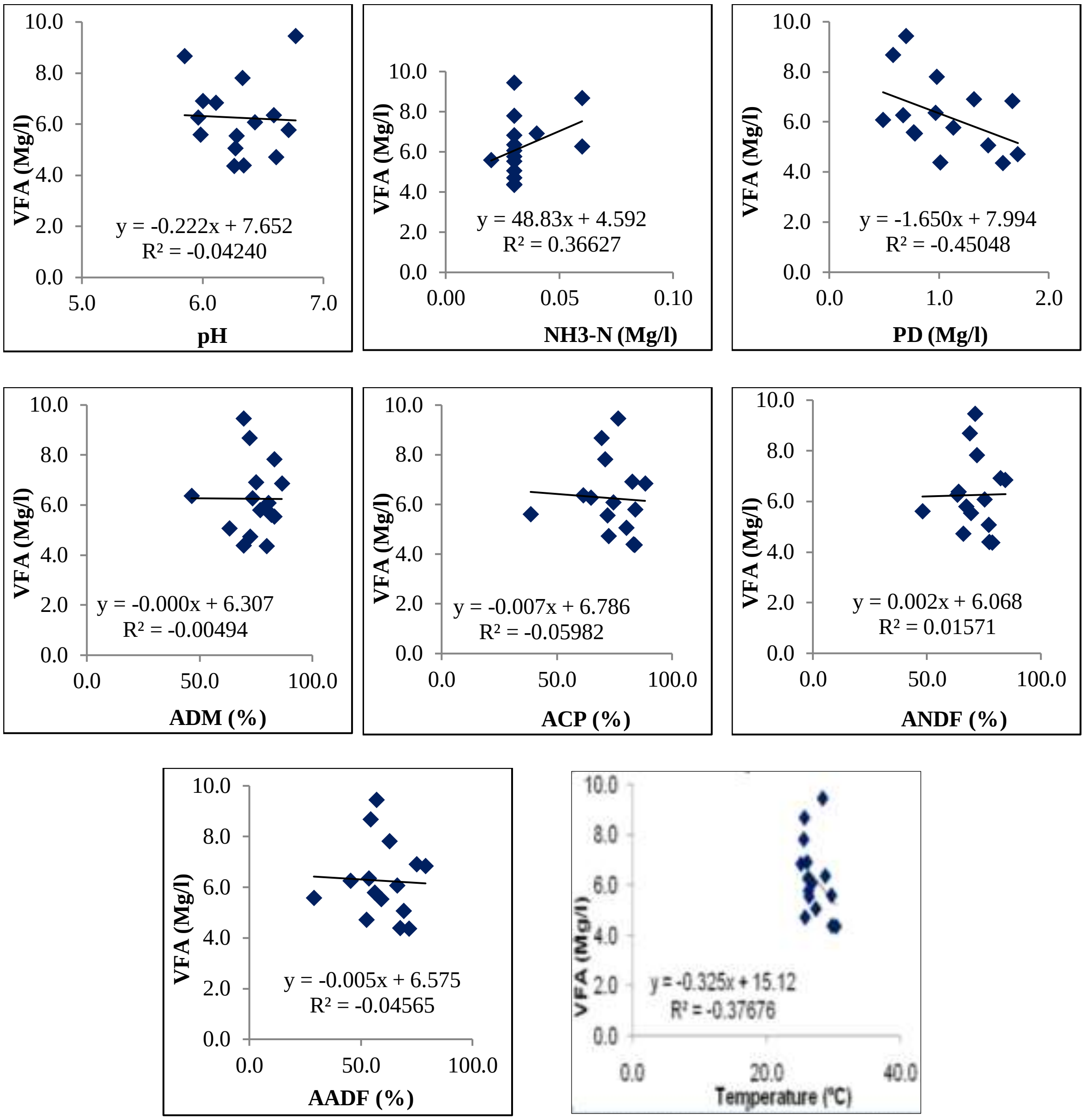

Figure 1: Pearson correlation coefficients of volatile fatty acids with rumen condition and digestibility of mature female goats

3.3 Correlation Values of Purine Derivatives with other Parameters on Rumen Condition and Apparent Digestibility

The effect of particular diets on microbial protein production can be estimated by the method based on measurement of purine derivatives (PD) in urine. Nucleic acids leaving the rumen are essentially of microbial origin. This is because ruminant feeds usually have a low purine content, most of which undergo extensive degradation in the rumen as the result of microbial fermentation. Absorbed nucleic acid purines are degraded and excreted in the urine as their derivatives, hypoxanthine, xanthine, uric acid and allantoin. The excretion of the PD is directly related to the purine absorption. With the knowledge of the purine-N: total-N ratio in microbial biomass, microbial N absorption can be calculated from the amount of purine absorbed which is estimated from urinary PD excretion. It is simple since it will just require total collection of urine and considered non-invasive since it does not require any surgical preparation of the animal (1). The relationship of purine derivative on different rumen condition parameters and apparent nutrient digestibility (DM, CP, NDF, and ADF) as presented in figure 3. Data showed that purine derivatives have moderately positive association with apparent nutrient digestibility (CP, NDF and ADF) but moderately negative relationship observed with VFA and NH3-N. The rumen temperature and pH as well as dry matter apparent digestibility showed negatively low relationship with purine derivatives.

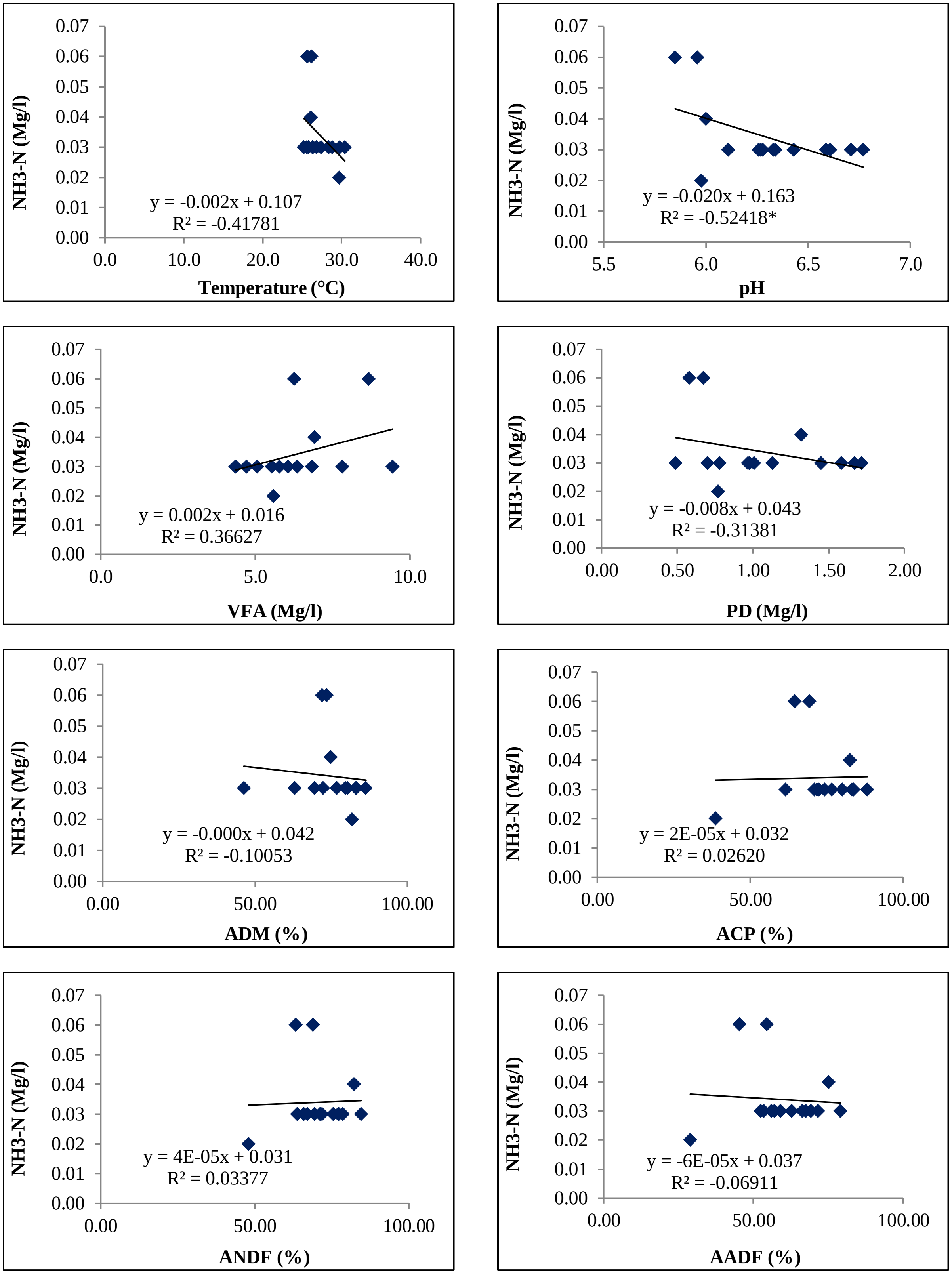

Figure 2: Pearson correlation coefficients of NH3-N with rumen condition and digestibility of mature female goats

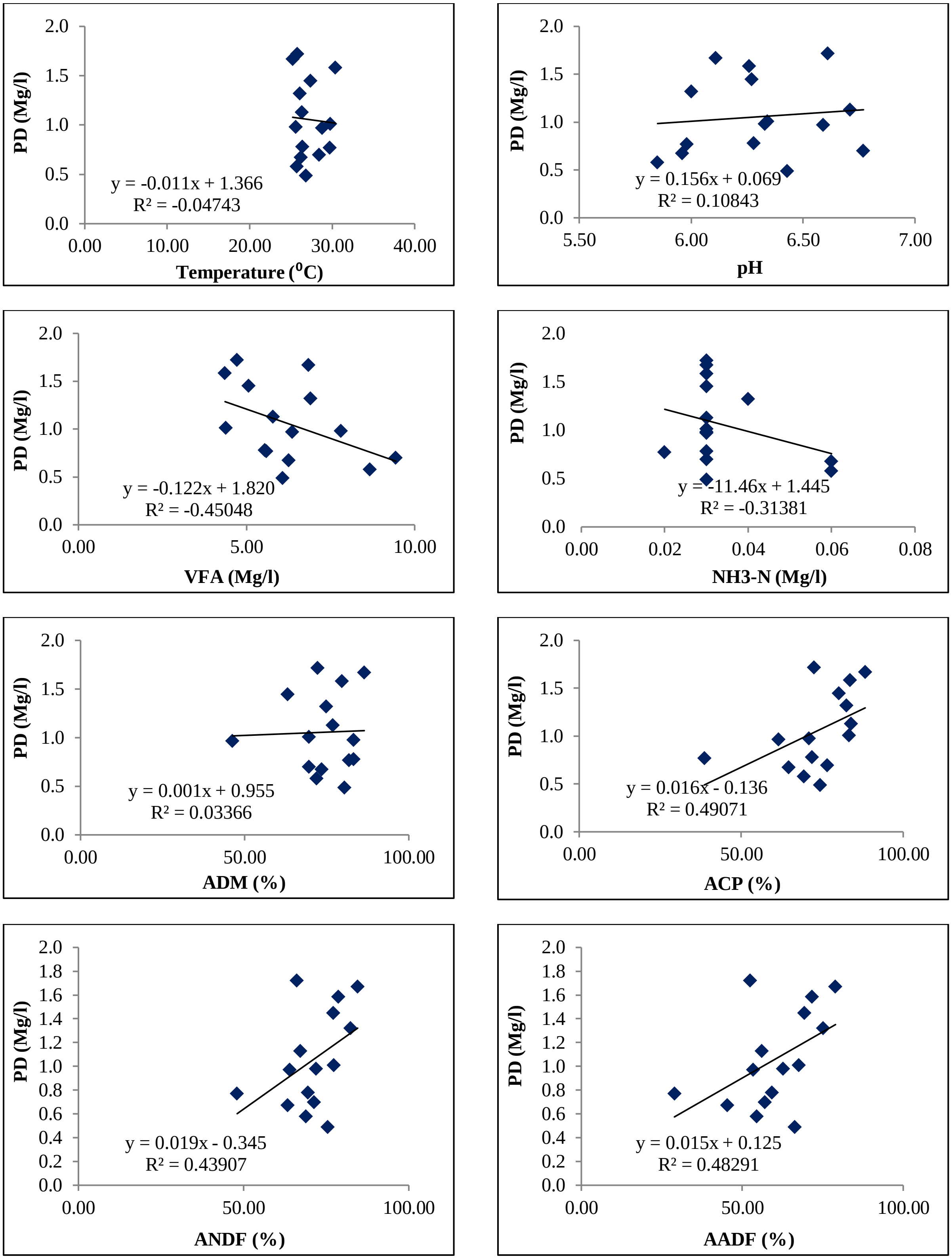

Figure 3: Pearson correlation coefficients of purine derivatives with rumen condition and digestibility of mature female goats

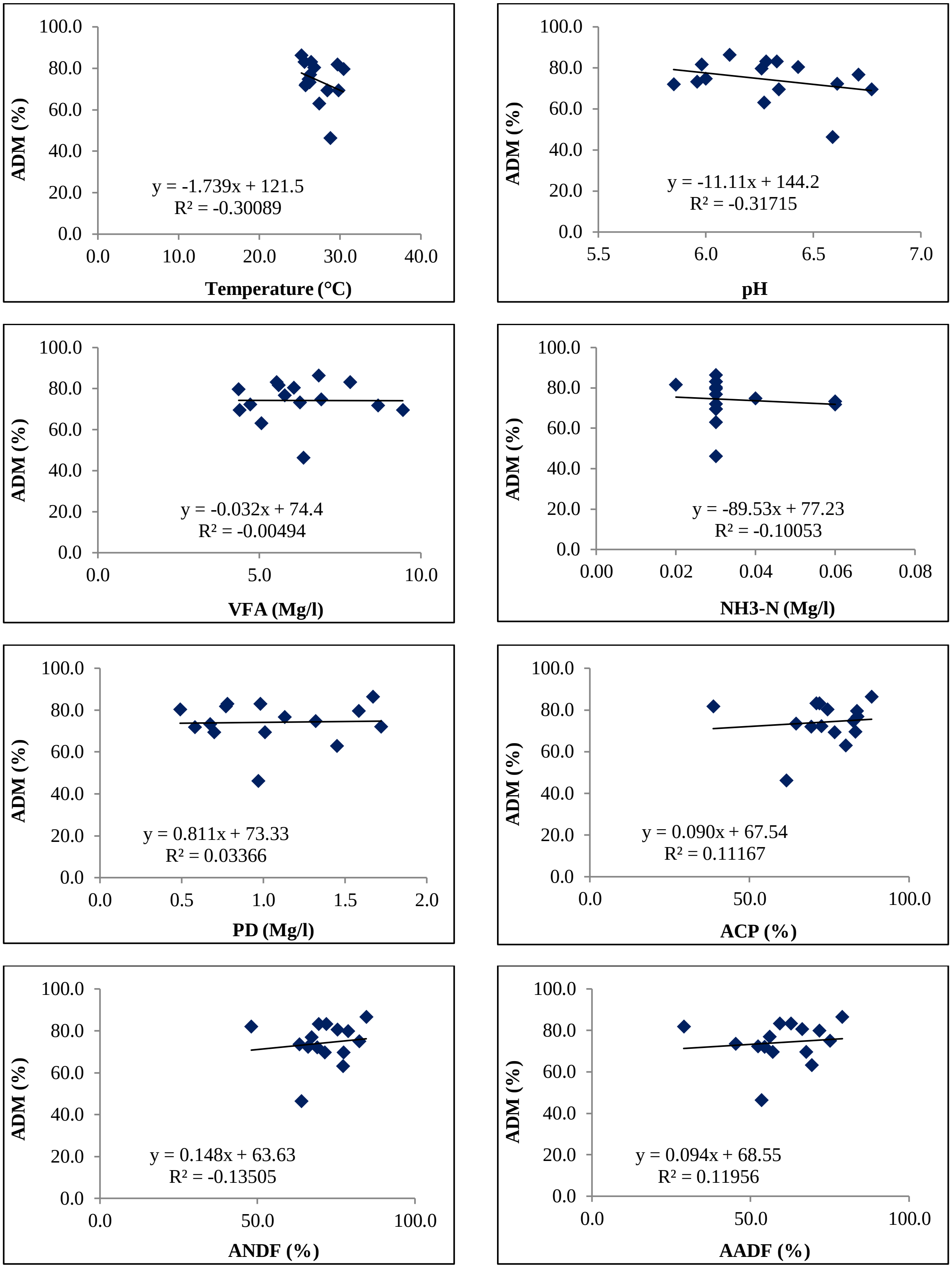

Figure 4: Pearson correlation coefficients of apparent dry matter with rumen condition and digestibility of mature female goats

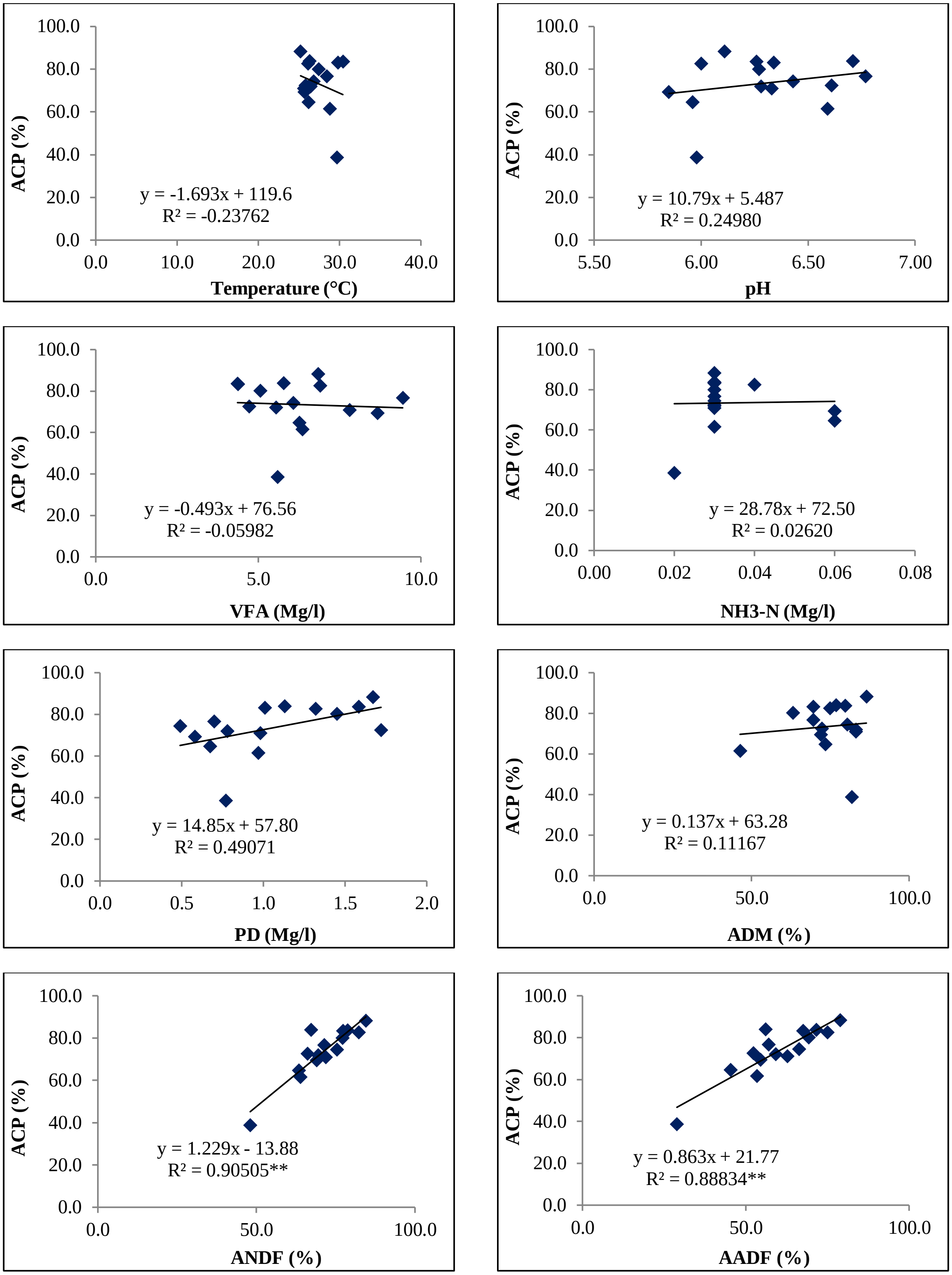

Figure 5: Pearson correlation coefficients of apparent crude protein with rumen condition and digestibility of mature female goats

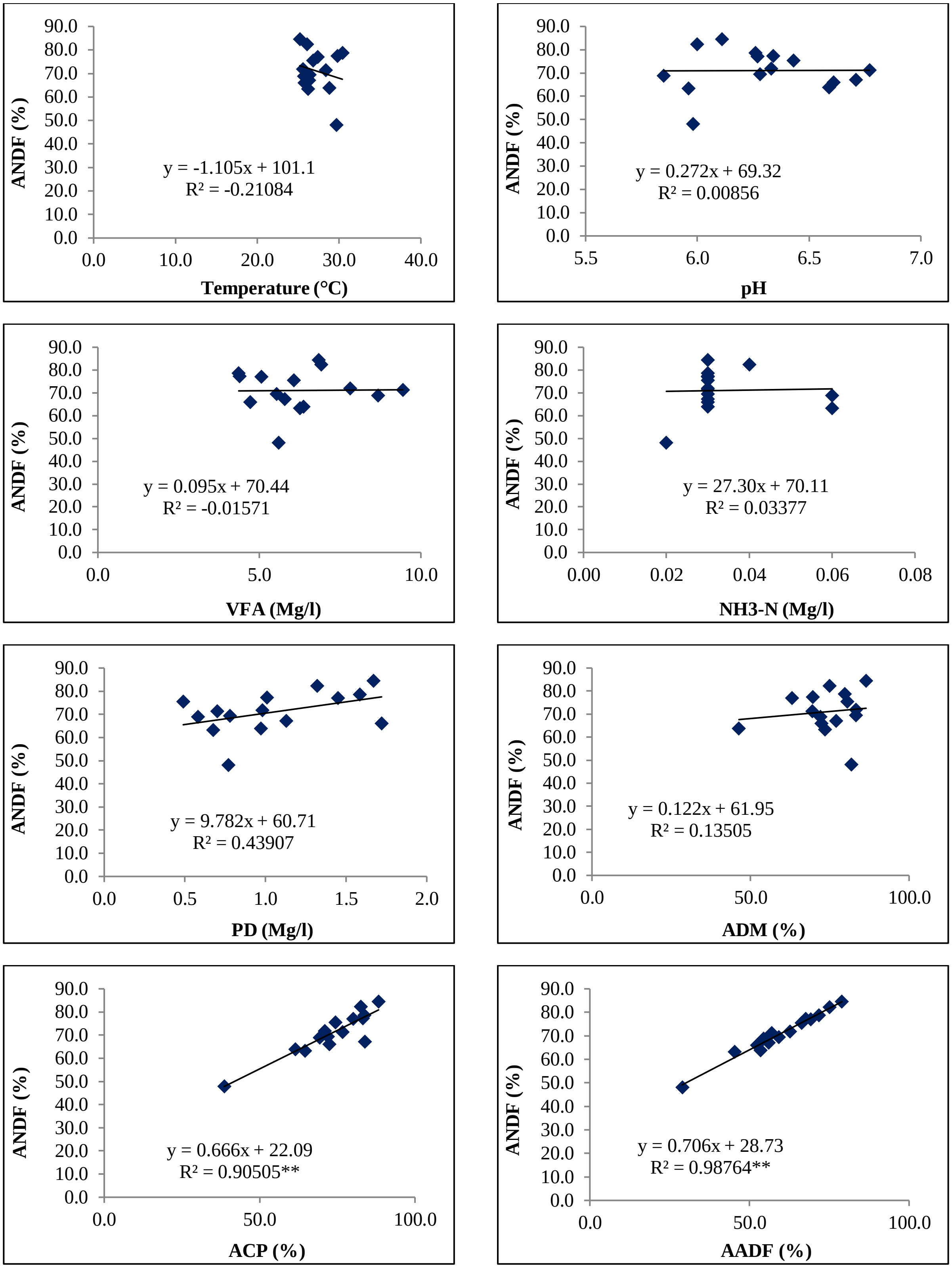

Figure 6: Pearson correlation coefficients of apparent neutral detergent fiber with rumen condition and digestibility of mature female goats

3.4 Correlation Values of Dry Matter Digestibility with other Parameters on Rumen Condition and Apparent Digestibility

Evaluating relationship of different parameters was analyzed in order to evaluate on how a specific parameters influenced the other parameters. This could provide idea to consider other parameters when research was conducted. The relationship of apparent dry matter digestibility on different rumen condition parameters (temperature, pH, NH3-N, purine derivatives and VFA) and apparent nutrient digestibility (CP, NDF, and ADF) as presented in Figure 4. Data showed that apparent DM digestibility have moderately positive association with the rest of apparent nutrient digestibility (CP, NDF and ADF) and negatively low association with the rest of rumen condition parameters (temperature, pH, NH3-N, purine derivatives and VFA).

3.5 Correlation Values of Crude Protein Digestibility with other Rumen Fluid Analyses and Apparent Digestibility

The correlation of apparent crude protein digestibility with other parameters on goat supplemented with different dietary fats (Figure 5) showed negatively low relationship with rumen temperature and VFA. However, positively low to moderate relationship were observed on rumen pH, NH3-N, apparent dry matter digestibility and purine derivatives.

Apparent crude protein digestibility was observed positively high association with apparent neutral and acid detergent digestibility. The data showed that and increase of 1% CP digestibility, there were 90 and 88% probability to increase in neutral and acid detergent digestibility, respectively.

3.6 Correlation Values of Nuetral Detergent Fiber Digestibility with other Rumen Fluid Analyses and Apparent Digestibility

Detergent fiber analysis indicates the concentration of fiber (hemicelluloses, cellulose, lignin and silica) in a sample. Acid detergent fiber indicates the concentration of cellulose, lignin and silica which compose the residue with hemicelluloses (neutral detergent fiber) dissolved in the ADF solution.

The relationship of NDF digestibility on different rumen condition parameters (temperature, pH, NH3-N, purine derivatives and VFA) and apparent nutrient digestibility (DM, CP and ADF) as presented in Figure 6. Data showed that apparent NDF digestibility negatively low relationship on temperature and VFA. However, low to moderately positive relationship were observed on apparent DM digestibility, rumen pH, NH3-N and purine derivatives. Perfectly positive relationship was observed on ADF digestibility. This means that, an increase of 1 % in apparent NDF digestibility, there is 98% possibility on increase in apparent ADF digestibility.

4 Conclusion

Generally, supplementing dietary fats to the concentrate of goat showed decrease protozoa population that ingest rumen bacteria resulted to increase digestibility on the supplemented groups which can be associated to the positively high associations in all nutrient apparent digestibility parameters while there were negatively high association between rumen pH and NH3-N. Moderately positive associations were observed between purine derivatives and apparent nutrient digestibility (CP and ADF). However, moderately negative relationship between VFA and purine derivatives and between NH_3-N and rumen temperature.

5 Recommendation

Due to positively high associations in all nutrient apparent digestibility parameters and between purine derivatives and apparent nutrient digestibility, fat supplementation indeed is a good feeding regimen to improved nutrient digestibility and hence recommended for utilization at farmers level. On the other hand, due to negatively moderately to high association between rumen pH and NH_3-N, VFA and purine derivatives and NH_3-N and rumen temperature, rumen ecology must be acidic (pH of 4-6), to reduced production of ammonia nitrogen and other GHG gases that causes climate change and global warming.

References

1. Chen XB, Gomes MJ. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details. Rowett Research Institure; 1992.
2. Chesworth J. Ruminant Nutrition Dietary Functions, 1992:93-99.
3. Coffey L, Hale M, Wells A. Goats: Sustainable Production Overview. Livestock Production Guide. NCAT Agriculture Specialists. IP248, 2004.
4. Ismail AI, Arafat SM. Quality Characteristics of High-Oleic Sunflower Oil Extracted from Some Hybrids Cultivated Under Egyptian Conditions. Journal of Food Technology Research, 2014;1(2):73-83.
5. Kanwar SS, Devi S. Thermostable xylanases of microbial origin: recent insights and biotechnological potential. The International Journal of Biotechnology, 2012;1(1):1-20.
6. Kareem SD, Olusegun AK, Samuel OO. The Effect of Co2 Emission and Economic Growth on Energy Consumption in Sub Sahara Africa. International Journal of Sustainable Energy and Environmental Research, 2017;6(1):27-35.
7. Kaygisiz F, Sezgin FH. Forecasting Goat Milk Production in Turkey Using Artificial Neural Networks and Box-Jenkins Models. Animal Review, 2017;4(3):45-52.
8. Kazora AS, Mourad KA. Assessing the National Sanitation Policy in Rwanda. Review of Environment and Earth Sciences, 2018;5(2):55-63.
9. Khan I. The Impact of Land Use on Spatial Variations of Begging in District Lahore_Pakistan. International Journal of Geography and Geology, 2018;7(2):27-34.
10. Malinga NG, Masuku MB, Raufu MO. Comparative analysis of technical efficiencies of smallholder vegetable farmers with and without credit access in swazil and the case of the Hhohho region. International Journal of Sustainable Agricultural Research, 2015;2(4):133-145.
11. Mohammed U, Olaleye RS, Umar IS, Ndanitsa MA, Jibrin S. Farmers Assessment of the Training and Visit Extension System in Niger State: Evidence from Fadama Ii & Iii in Mokwa Local Government Area of Niger State, Nigeria. International Journal of Sustainable Agricultural Research, 2015;2(4):111-119.
12. Mukadasi B. Mixed Cropping Systems for Sustainable Domestic Food Supply of the Smallholder Farming Communities in Nakasongola District, Central Uganda. Canadian Journal of Agriculture and Crops, 2018;3(1):42-54.

13. Navaeefard A, Babaee O, Radmanesh H. Optimal sizing of hybrid systems and economical comparison. International Journal of Sustainable Energy and Environmental Research, 2017;6(1):1-8.
14. Roffler RE, Satter LD. Relationship Between Ruminal Ammonia and Nonprotein Nitrogen Utilization by Ruminants. I. Development of a Model for Predicting Nonprotein Nitrogen Utilization by Cattle1. *Journal of Dairy Science*, 1975;*58*(12):1880-1888.
15. Roffler RE, Satter LD. Protein Requirement and Non Protein Nitrogen Utilization. *Journal of Dairy Science*, 1975;*58*(12):1880-1889.
16. Roxas DB. Animal Feeds and Feeding: With Emphasis on the Tropics. pp.1. University of the Philippines Los Baños, Laguna, Philippines: University Publication Office, 2006.
17. SAS institute Inc., 2003. SAS User's Guide, Version 9.1, second ed. SAS Institute Inc. Cary, NC.
18. Stern MD, Bach A, Calsamiglia S. New concepts in protein nutrition of ruminants. In21st Annual Southwest Nutrition & Management Conference 2006 Feb 23 (pp. 45-62).