[1]Bahram Chacher, [2] Jian-Xin Liu [2,] Hong-Yun Liu and [1] Illahi Bakhsh Marghazani

drbahram_vet@hotmail.com*

(Received on 21st July 2014, accepted in revised form 2nd December 2014)

The objective of current study was to build up a convenient, economic and accurate procedure to determine arginine (ARG) concentration in rumen fluid. Rumen fluid was collected from 3 rumen fistulated Chinese Holstein dairy cows and added with or without (control) 1mmol/l unprotected ARG and blank (with only medium) in to syringe system in triplicate as a replicate. All syringes were incubated in water bath at 39 ℃ for 0, 2, 4, 6, 12 and 24 h and were terminated to measure the ARG concentration. Sakaguchi reaction method was used to analyze the ARG concentration in rumen fluid by determining the rumen degradation rate of protected and unprotected ARG. Temperature, time and absorbance were optimized in the procedure based on Sakaguchi reaction. Color consistency remained 4-6 min. The optimum temperature (0-5) ℃ was observed for maximum optical density 0.663 at wave length 500 nm. Minimum ARG that could be determined in rumen fluid by spectrophotometer was 4-5 µg/ml. No significance ($P>0.05$) difference were observed between two results derived from spectrophotometer and amino acid analyzer methods. In conclusion, the spectrophotometer method of ARG determination in rumen fluid based on Sakaguchi reaction is easy, accurate, and economical and could be useful in learning ARG metabolism in the rumen.

Key words: Arginine; Rumen fluid; Sakaguchi reaction; Spectrophotometer, Method development.

Arginine (ARG) is well recognized amino acid (AA) for its importance in urea cycle and for precursor of polyamines and nitric oxide. Previous reports showed that abomasal administration of ARG enhanced milk production in dairy cows [1], improved the nitrogen metabolism in heifers [2], and enhanced the immunity and growth performance of pre-ruminant calves [3]. Recently, injection of ARG-HCl was reported to decreased embryonic loss in ewes [4], increased lamb birth weight in gestationally nutrient restricted ewes [5], and improved fetal lamb survival to term in prolific ewes [6]. Parental administration of L-Arg increased the brown adipose tissue in lambs and enhanced neonatal thermogenesis at birth [7]. However parental and abomasal administration of ARG to farm animal is not practical, due to complexity of ARG metabolism in ruminant animals. Therefore, determination of ARG concentration in rumen fluid to understand the rumen degradation, fermentation and intestinal digestibility, will be an essential step before feeding.

Regular methods used for ARG determination in rumen fluid include high performance liquid chromatography (HPLC) [8] and AA analyzer [9]. The use of HPLC and AA analyzer were of high sensitivity and accuracy, but were of high cost, time-consuming, and were not commonly available to small-scale animal nutrition laboratories. Whereas, use of spectrophomter for ARG determination in biological fluid with Sakaguchi reaction is relatively simple, cheap and sensitive [10, 11], but was not used in rumen fluid. Thus, a procedure based on Sakaguchi reaction for the determination of ARG in rumen fluid should be developed for small scale animal nutrition laboratories to study the ARG metabolism in rumen and for intestinal digestibility.

Commercial unprotected ARG with purity 98.5% the rumen protected ARG 60% with 40% oil palm layer. 5-Sulfosalicylic acid. Bromine purity 99.5%. Hydorxyquinoline (8-Quinolinol) with 99% purity, Urea purity ≥98%. Sodium Hydroxide NaOH 96% purity and Ethanol purity 99.7% were used for the determination of ARG in rumen fluid. Distilled water was purified by a Millipore system MilliQ.

For rumen unprotected, Calibrated glass syringes (Model Fortuna, Häberle Laborte –chnik,

Lonsee-Ettlenschieß, Germany) were used as incubation. The oven dried substrate 200 mg dry matter (DM) basis Chinese wild grass and corn meal with ratio of 50:50 were weighed into 100 ml glass syringes in triplicate. Rumen fluids were collected from three ruminally reviously fed the

concentrations of 8-ydroquinoline and sodium hydrobromite. Temperature, time and absorbance were considered as important factors.

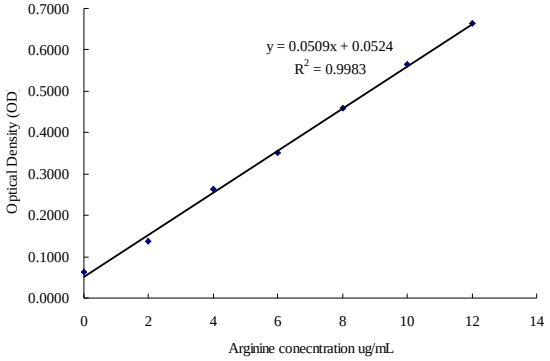


Fig. 1: Standard line at different Arginine concentration

The 1mmol/l ARG in rumen fluid determined by Spectrophotometer was calculated by subtracting the concentrations of ARG µg/ml in the control and blank samples as method applied by Chacher [13]. The concentration of ARG in rumen fluid by AA analyzer was estimated according to method described by Broderick and Kang ([9]). The data of ARG concentration in rumen fluid obtained by two methods were analyzed with one way analysis of variance using PROC GLM (General Linear Model) procedure of SAS [17] package. Results were subjected to multiple range tests, at <0.05 and standard error of means (SEM).

In the current study Sakaguchi method was used to analyze the ARG concentration in rumen fluid by determining the rumen degradation of ARG.

Effect of Temperature and Time on Colour Development and Determination Stability

The effect of temperature on colour development and determination stability was recorded as average OD values 0.6635 ± 0.0046 (SD), 0.6630 ± 0.0033, 0.6308 ± 0.0053 and 0.5901 ± 0.0040, with loss of 0.00, 0.03, 4.92 and 11.06% for 0, 5, 10 and 15 °C, respectively, at 12 ARG concentrations.

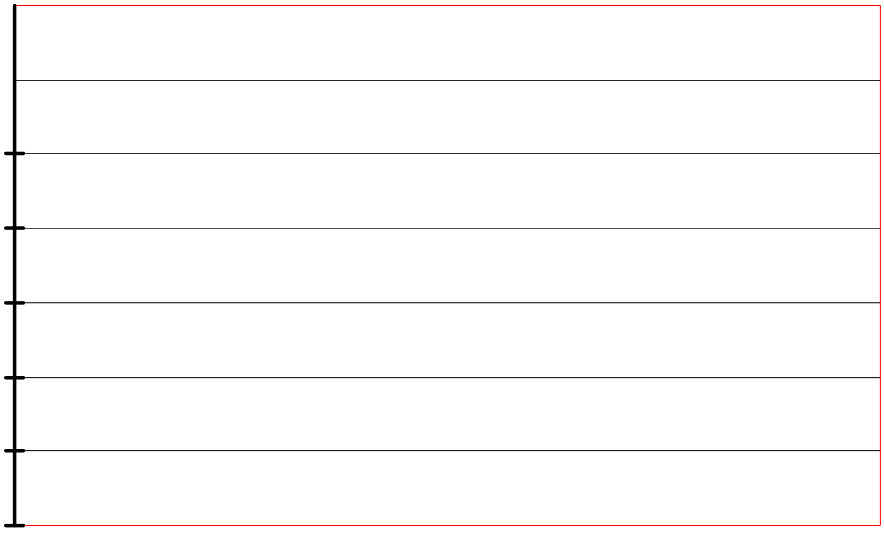
The observed OD valued for color development and consistencies by time were recorded as 0.6633 ± 0.0035 (SD), 0.6610 ± 0.0044, 0.6450 ± 0.0055, 0.5957 ± 0.0105, with loss of 0.00, 0.35, 2.71 and 10.20 % within 0, 5, 10 and 15 min, and respectively.

The temperature was one of the most critical factor affecting the colour development and stability. The results indicated negative co-relationship (= -0.093) between temperature and OD values (Fig 2). In current study maximum OD (0.663) was observed in the range of 0-5°C, when the ARG concentration was 12 , while Ceriotti and Spandrio [12] reported an optimized with maximum OD (0.80), at 8 l ARG concentration and tempreture of 10-15°C. The reasons of optimum temperature 10-15°C and OD (0.80) value obtained by Ceriotti and Spandrio, [12] at lower ARG concentration (8 l) might be due to addition of butanol also differences in wave length and instrument used. Moreover, excess urea used instead of butanol and samples were stabilized on ice. It was tested that addition of butanol ended a complexity and has drawback to get samples from mixture that ultimately affect the linearity. In the present study ARG determination without butanol was proved to be easy and accurate for Sakaguchi reaction.

The color developed by the reaction of ARG with 8-hydroxyquinoline in rumen fluid was stabilized by addition of excessive urea on ice instant. No decreases in color were observed within 4 min. Colour began to decrease after 5, 10 and 15 min with loss of 0.35, 2.71 and 10.20 %, respectively. It was indicated that the values should be obtained with in 4-6 min after development of color (Fig. 2). The reported research showed maximum time for colour stability was about 3-4 min [10]. The difference in results between the current study and the studies conducted by Ceriotti and Spandrio, [12] and Wang ., [10] might be due to difference in experimental procedure.

The results of selection of absorbance tested at different WL were recorded as OD value of 0.0010, 0.0500, 0.1000, 0.1570, 0.2500, 0.3500, 0.4500, 0.5350, 0.5985, 0.6451, 0.6633, 0.6100, 0.4980, 0.2500, 0.0004 and 0.0000, for WL 350, 365, 380, 395, 410, 425, 440, 455, 470, 485, 500, 515, 530, 545, 560 and 575 nm, respectively.

The optimized concentrations of sodium hypobromite and 8-hydroquinoline for ARG quantitative determination were 0.4 and 0.02 % respectively. In the present study 8-hydroxyquinoline was selected because chromomeric product formed had much higher apparent molar absorption and the OD values than those with other Sakaguchi reagents. The WL 500 nm which had maximum OD value of (0.663) (Fig. 2) was selected for ARG quantitative analysis in the rumen fluid based on Sakaguchi reagents, which was consisted with study conducted by Wang [10].



fluid with Sakaguchi reaction through spectrophotometer.

This research was financially supported by China Agriculture Research System (No. CARS-37). The work was conducted at Institute of Dairy Sciences, MoE Key laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University Hangzhou, 3100058.

1. B. P. Chew, J. R. Eisenman and T. S.Tanaka, Arginine Infusion Stimulates Prolactin, Growth Hormone, Insulin and Subsequent Lactation in Pregnant Dairy Cows, , 2507 (1984).
2. G. M. Davenport, J. A. Koling, K. K. Schillo and D. K. Aaron, Nitrogen Metabolism and Somatotropin Secretion in Lambs Receiving Arginine and Ornithine Via Abomasal Infusion, , , 222 (1990).
3. J. M. Fligger, C. A. Gibson, L. M. Sordillo and C. R. Baumrucker, Arginine Supplementation Increases Weight Gain, Depresses Antibody Production and Growth Hormone Concentrations and Alters Circulating Leukocyte Profiles in Preruminant Calves without Affecting Plasma Growth Hormone Concentrations, , , 3019 (1997).
4. J. S Luther, E. J. Windorski, C. S. Schauer, J. D. Kirschi, K. A. Vonnahme, L. P. Reynolds, J. S. Caton and G. Wu, Impacts of l-arginine on Ovarian Function and Reproductive Performance in Ewes, , (2008).
5. A. Lassala, F. W. Bazer, T. A. Cudd, D. Datta, D. H. Keisler, M. C. Satterfield, T. E. Spencer, and G. Wu, Parenteral Administration of L-arginine Prevents Fetal Growth Restriction in Undernourished Ewes. , 1242 (2010).
6. A. Lassala, F. W. Bazer, T. A Cudd, S. Datta, D. H. Keisler, M. C. Satterfield, T. E. Spencer and G. Wu, Parenteral Administrtion of L-arginine Enhances Fetal Survival and Growth in Sheep Carrying Multiple Fetuses, , 849 (2011).
7. M. C. Satter eld, K. A. Dunlap, D. H. Keisler, F. W. Bazer andG. Wu, Arginine Nutrition and Fetal Brown Adipose Tissue Development in Diet-Induced Obese Sheep, , , 1593 (2012).
8. H. Sultana, R. Onodera, M. M. Or-Rashid and S. Wadud, Convenient Method for the Determination of Arginine and Its Compounds in Rumen Fuid by Reversed-phase high-performance Liquid Chromatography, , B, 321 (2001).
9. G. A. Broderick and J. H. Kang. Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and in Vitro Media, , 4 (1980).
10. H. Wang, X. H. Liang, R. X. Zhao, L. D. Feng and H. Li, Spectrophotometer Determination of Arginine in Grape Juice Using 8-hydroquinoline, , 1210 (2008).
11. H. Li, X. H. Liang, Li. Feng and H. Wang, Determination of L-arginine Content in Fermentation Liquor by Thymol-Spectrophotometer Method, , 26 2007).
12. G. Ceriotti and L. Spandrio, An Improved Method for The Micro Determination of Arginine by use of 8-hydroxyquinoline, 603(1957).
13. B. Chacher D. M. Wang, H. Y. Liu and J. X Liu, Degradation of L-arginine and N-carbamoyl Glutamate and Their Effect on Rumen Fermentation in Vitro, , 374 (2012).
14. K. H. Menke, and Steingass, Estimation of The Energetic Feed Value Obtained From Chemical Analysis and In vitro Gas Production Using Rumen Fluid, , 7, (1988).
15. D. M. Wang, H. Y. Liu, C. Wang and J. X. Liu, Preparation and Stability of Rumen Protecte - amino butyric acid, , , 1451 (2010).
16. D. D. Gilboe and J. N. William, Evaluation of the Sakaguchi Reaction for Quantitative Determination of Arginine, , , 535 (1956)
17. Statistical Analysis System Institute Inc., Version 8.0. SAS Inst Inc, Cary, NC, USA. (1999)