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EFFECT OF TANNIC ACID ON THE DEGRADATION KINETICS OF PROTEIN AND ORGANIC MATTER OF ALFALFA HAY

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ABSTRACT

The effect of tannic acid on the degradation kinetics of protein and organic matter (OM) of alfalfa hay was studied using four ruminally-cannulated sheep under feeding oats hay as a sole diet. Alfalfa hay was treated with tannic acid (TA) at four different levels, which were alfalfa hay +0% of TA (control; T0), alfalfa hay +TA at 1.5% of DM (T1.5), alfalfa hay +TA at 3.0 % of DM (T3.0) and alfalfa hay +TA at 4.5% of DM (T4.5).

Inhibition effect of TA on the disappearance of protein was significant ($P<0.05$) up to 12 h of incubation for T4.5, however this effect was noticed up to 6 h for T1.5 and T3. Tannic acid through the first 3h of incubation did not affect disappearance of OM in alfalfa hay.

The highest level of TA (T 4.5) had significantly reduced ($P<0.05$) OM disappearances at incubation times of 6 and 12h. No significant effect was found for TA on the disappearance of OM at the incubation times 24 and 48h.

The amount of protein which was slowly degraded in the rumen was significantly increased ($P<0.05$) in treated hay. The increasing of TA consistently decreased the degradation rate (*c*) of protein.

Tannic acid did not affect rapidly (*a*) and slowly (*b*) degraded fractions of OM, however, the *c* of OM was significantly decreased ($P<0.05$) at T3.0 and T4.5.

These results suggested that lower level of TA (i.e. T1.5) appears to have a desirable reduction effect on protein disappearance without shortening energy release from OM.

Keywords: Alfalfa hay, degradation kinetics, tannic acid, protein, organic matter

INTRODUCTION

Soluble protein intake is the portion of dietary intake protein that is immediately available for bacterial metabolism in the rumen. The rate at which dietary intake protein is degraded in the rumen can affect the amount of ammonia-N (NH_3-N) that escapes microbial capture, depending on the availability of readily fermentable carbohydrate sources to provide ATP in support of microbial protein (Hoover and Stokes, 1991). If there is insufficient rumen-available energy or the degradation rate of nitrogen (N) and energy are not synchronized, the excess NH_3-N will be absorbed into portal blood and transported to the liver where it is converted to urea (Hoover and Stokes, 1991). Depending on the prevailing dietary condition, 40-60% of liver urea output is excreted in urine (Huntington, 1989), which represents an irretrievable loss of N to the animal and is considered a sources of pollution.

Legume forage has a higher protein concentration with a fast degradation rate and higher portion of soluble protein (Kamel *et al.*, 1995 and El-Waziry *et al.*, 2000). In addition, NH_3-N concentration of rumen liquor was raised up to 8.28

m*M* when sheep were fed berseem hay as a sole diet (Kamel *et al.*, 2000), is 3 folds higher than the minimum level-recommended by Satter and Slyter (1974) for microbial protein synthesis. Sinclair *et al.*, (1995) concluded that optimum microbial protein synthesis was achieved when the ratio between hourly release of nitrogen and organic (OM) matter was 25 g N/ kg of OM ruminally degraded. However, in a pervious study this ratio was found to be 34.5 g N/ kg OM, when sheep were fed berseem hay as a sole diet (unpublished data).

It should be pointed out that optimum NH_3-N concentration for maximal rate of fermentation was found to be much higher than that required for maximum microbial protein synthesis/unit of energy, being dependent on roughage: concentrate ratio in the basal diet (Mehrez, 1992).

Tannins are complex phenol-rich polymers found in many foods. The anti-nutritive effects of tannins are associated with their ability to combine with dietary protein thus reducing its digestion. The objective of the current study was to evaluate the effect of different levels of tannic acid on the degradation kinetics of alfalfa protein and OM in the rumen.

MATERIAL AND METHODS

Animals and feed

Four ruminally cannulated Suffolk sheep (77.5±1.7 kg, BW) were used in a 4X4 Latin square design experiment. Sheep were offered oats hay *ad lib.*, and they had free access to fresh water. Animals were kept in individual cages at the Experiment Station of Tottori University, Japan. Tested alfalfa hay was treated with tannic acid (TA, Wako Pure Chemical Industries, Ltd., Japan) at four different levels. Treatments were alfalfa hay +0% of TA (control; T0), alfalfa hay + TA at 1.5% of DM (T1.5), alfalfa hay + TA at 3.0 % of DM (T3.0) and alfalfa hay + TA at 4.5% of DM (T4.5). Tannic acid was dissolved in water (20%, v/w) and applied as an aqueous spray to the hay, then thoroughly mixed. Treated alfalfa hay was dried in an oven for 24h at 50 °C.

Organic matter (OM) and crude protein (CP) in oats hay and alfalfa hay was determined according to the AOAC (1990). Neutral detergent fiber (NDF) was determined as described by Goering and Van Soest (1970). Chemical composition of given hay was 91.6, 9.7 and 63.5 for OM, CP and NDF, respectively.

In situ degradation kinetics of OM and protein of alfalfa hay

In situ technique was done as outlined by Meherz and Ørskov (1977). Disappearances of OM and N were determined by incubating 6 g (ground to 2 mm) of air-dry alfalfa hay in nylon bags, then bags were placed in the rumen. The bags measured 200 X 100 mm and made from polyester cloth (Swiss Nylon Monofilament, Switzerland) with pore size of 50 µm. Ten bags were inserted into the rumen of each animal through the cannula, then 2 bags were withdrawn at each incubation time (3, 6, 12, 24 and 48h). After withdrawal from the rumen, bags were washed under running tap water until the water became clear, then they were squeezed gently. Microorganisms attached to

the residual samples were eliminated by freezing-rethawing technique as descried by Kamel *et al.*, (1995a). The washing loss of N or OM was determined by washing 2 bags at each treatment in running water for 30 min. Bags were dried in oven for 48 h at 80°C. The degradation kinetics of OM and protein were estimated by fitting the disappearance values to the following exponential equation $P = a + b\ (1\text{-}exp^{-ct})$ as proposed by Ørskov and McDonald (1979), where P represents the disappearance at time t. The a, b and c are constants in the exponential equation, and are defined as the rapidly degraded fraction, slowly degraded fraction and rate of degradation, respectively. The lag time (L_t) was estimated according to McDonald (1981).

Statistical analysis

Data were analyzed by ANOVA for a 4x4 Latin square design using the JMP procedure of SAS Institute, Inc. (1994). Respective mean values were compared using Tukey/ Kramer test.

RESULTS

Chemical composition (%, on dry matter basis) of alfalfa hay was 89.05, 21.9 and 36.8 for OM, CP and NDF, respectively.

Disappearance of N as influenced by TA is presented in Figure 1. Washing loss of N was significantly reduced ($P<0.05$) when alfalfa hay was treated with TA at the level of T 3.0 and T 4.5, meanwhile T 1.5 had no effect. Values of N disappearance at time 0 h of incubation were 0.39, 0.37, 0.34 and 0.31 for T 0, T1.5, T 3.0 and T 4.5, respectively. Alfalfa hay treated with TA had lower ($P<0.05$) N disappearance compared to the control at incubation time of 3 and 6 h (Figure 2). Reduction of N disappearance was significantly lower ($P<0.05$) at T1.5 and T3.0 than T4.5 at both incubation times 3 and 6h. The inhibition effect of TA on the N disappearance was noticed up to 12 h of incubation for level T4.5, however that in levels T1.5 and T3.0 had no significant effect. Nitrogen disappeared from nylon bags tended to be lower at incubation time 24h for T1.5, T3.0 and T4.5 compared with the control. The values of N disappearance at 48h were 0.93, 0.94, 0.93 and 0.92 for T0, T1.5, T3.0 and T4.5, respectively, with no significant difference among the treatments (Figure 1).

Figure 2 illustrates the effect of different levels of TA on the disappearance of OM in alfalfa hay, using *in situ* technique. No significant differences were found due to TA during incubation times 0 and 3h, however, TA has numerically reduced OM disappearances compare to the control. At the incubation time of 6 and 12h, TA at the level of 1.5 and 3.0% of DM did not affect disappearance of OM. Meanwhile, the highest level of TA (T 4.5) had significantly reduced ($P<0.05$) OM disappearances at both incubation times (6 and 12h). Also, the inhibition effect of TA was observed at incubation time of 24h, however, no significant differences were detected among tested levels. Extent of OM disappearance in alfalfa hay was not affected by TA and the values were 0.77, 0.78, 0.79 and 0.77 in T0, T1.5, T3.0 and T4.5, respectively.

Degradation kinetics of protein and OM in alfalfa hay is presented in Table 1. Rapidly degraded fraction (a) of protein had similar trend as

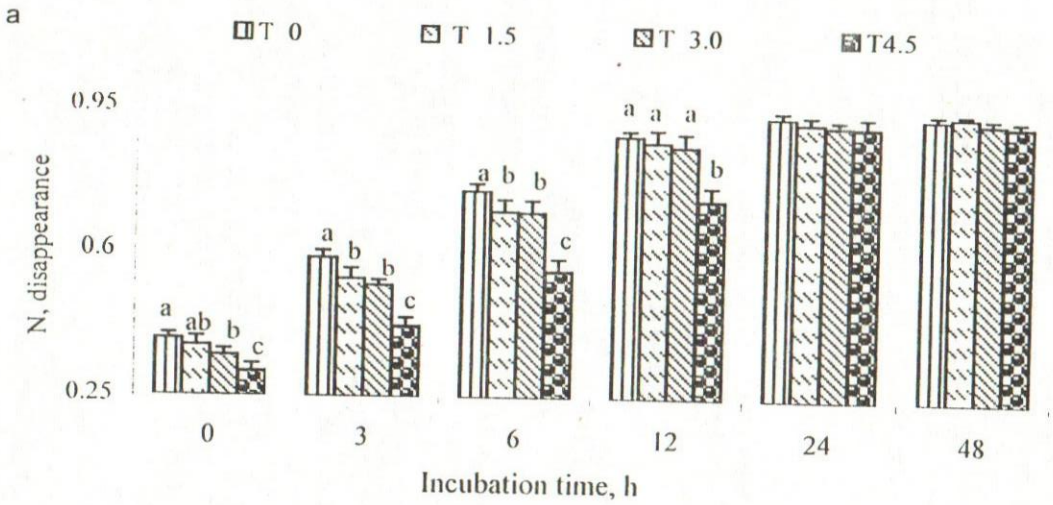

Figure 1. Effect of different levels of tannic acid on the disappearance of N in alfalfa hay, bars indicate standard errors. Column not sharing common superscripts at each incubation time differ *(P<0.05)*.

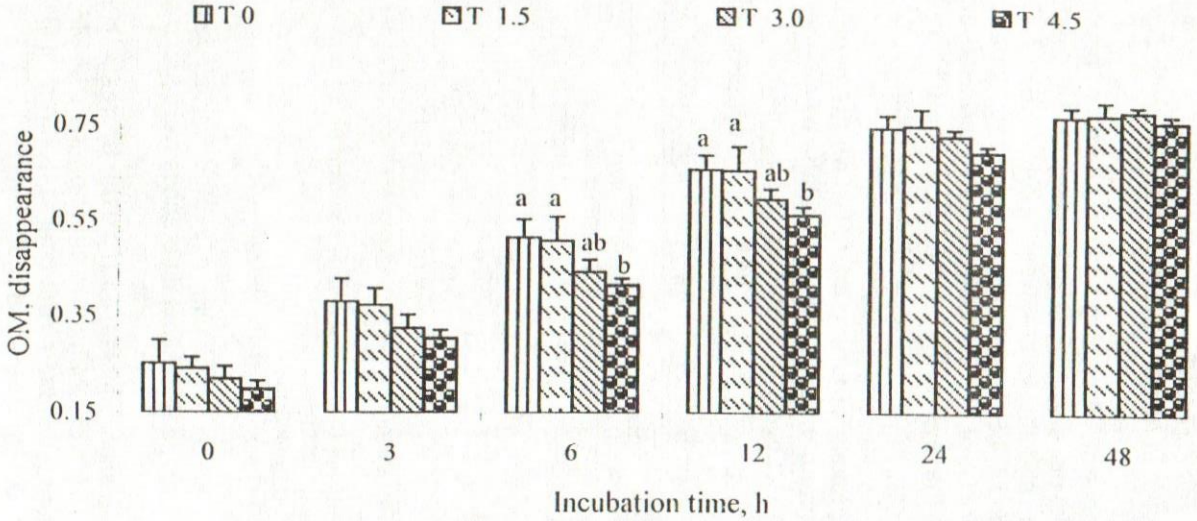

Figure2. Effect of different levels of tannic acid on the disappearance of OM in alfalfa hay, bars indicate standard errors. Column not sharing common superscripts at each incubation time differ *(P< 0.05)*.

obtained in N washing-loss, the levels T3.0 and T4.5 had significantly decreased ($P<0.05$) *a* fraction compared to control. However, this value in T1.5 had an intermediate value (Table 1). The *b* fraction of protein was significantly increased ($P<0.05$) in response to TA, meanwhile, no significant difference was found among TA treatments.

The suppression effect of TA on degradation rate (*c*) of protein was significant ($P<0.05$) at all levels of TA. The increasing of TA consistently decreased the *c* of protein, and the values were 0.208, 0.168, 0.138 and 0.101/h, for T0, T1.5, T3.0 and T 4.5, respectively. The lag time (time spent till protein started to disappear, L_t) of protein tended to be increased and this increment was significant at the level of T4.5 ($P<0.05$) compared with T0, no significant difference was found among treated alfalfa hay (Table 1).

Table 1. Effect of different levels of tannic acid (TA) on the degradation kinetics of protein and organic matter of alfalfa hay

	Levels of tannic acid*				
	T0	**T1.5**	**T3.0**	**T4.5**	**SEM**
Protein					
a	27.91^{z}	25.72^{zy}	24.21^{y}	23.41^{y}	0.83
b	65.89^{z}	68.71^{y}	70.71^{y}	71.05^{y}	0.61
c	0.208^{z}	0.168^{y}	0.138^{yx}	0.101^{x}	0.009
Lt	0.80^{z}	1.13^{zy}	1.19^{zy}	1.25^{y}	0.10
Organic matter					
a	18.35	16.39	16.41	15.45	1.81
b	58.60	61.61	62.81	61.1	1.34
c	0.137^{z}	0.128^{z}	0.099^{y}	0.0935^{y}	0.005
Lt	0.84	0.95	1.05	0.91	0.30

***Alfalfa hay +0% of TA (control; T0), alfalfa hay +TA at 1.5% of DM (T1.5), alfalfa hay +TA at 3.0 % of DM (T3.0) and alfalfa hay +TA at 4.5% of DM (T4.5)**

The *a*, *b* and *c* are constants predicted by the exponential equation $P= a + b\ (1\text{-}exp^{-ct})$ as proposed by Ørskov and McDonald (1979).

***Lt*= lag time, calculated as reported by McDonald (1981).**

$^{z\text{-}x}$ Means within a row with no common superscripts differ ($P<0.05$).

Rapidly degraded fraction (*a*) of OM in treated hay tended to be lower than control hay, however, no significant differences were found among the levels of TA.

Fraction *b* of OM has slightly increased ($P>0.05$) by treated hay with TA. Tannic acid at the level 1.5% of DM had no effect on the *c* of OM, but T 3.0 and T4.5 had significantly reduced ($P<0.05$) degradation rate of OM. The *c* values of OM were 0.137, 0.128, 0.099 and 0.0935/h for T0, T 1 .5, T3.0 and T4.5, receptively (Table 1). Tannic acid did not affect the L_t.

DISCUSSION

Although the degradation kinetics of alfalfa hay was done under feeding of oats hay, the potential extent of protein degradation (*a*+*b*) in T 0 %

was found to be 0.94, which resembled that obtained by Kamel *et al.*, (1995b), when sheep were fed alfalfa hay. Moreover, Kamel *et al.*, (1995c) reported that the protein degradation kinetics of alfalfa hay was not affected by feeding of either Italian ryegrass hay or Sudangrass hay as sole diet. Therefore, in the present study the degradation value of protein in alfalfa hay appears to be reliable when it was determined under feeding different hays as sole diets.

The amount of ammonia nitrogen produced as a measure of the proteolytic activity of rumen microorganisms was depressed when soybean meal protein was treated with tannic acid (Driedger and Hatfied, 1972). Forming hydrogen bonds between the phenolic sub-units of the polymer and carbonyle group of peptides of the protein result in a tannin-protein complexes which may protect protein from ruminal but not abomasal digestion because their stability can be pH-dependent (Barry and Manley, 1984). The complexing ability of tannins means that polyphenolics are reactive with the cell wall of bacteria and the extracellular enzymes secreted (McSweeney *et al.*, 2001). Results of N disappearance in this study support the general hypothesis that the low and moderate levels of tannins reduce the ruminal degradation of protein and hence reducing NH_3-N production.

Recently, Singh *et al.*, (2001) found that the gallic acid and pyrogallol (as a biodegraded products for tannic acid) were detected in the samples collected at 24 h of incubation with rumen liquor, and either of gallic acid or residual tannic acid could not be detected at 48 and 72 h. Odenyo and Osuji (1998) have cited three strains of a tannin-tolerating bacterium (*Selenomonas* sp.) from the rumen microflora of sheep and goats that had either been fed or had browsed tanniniferous forages. One of the strains (EAT2) of this ruminal bacterium could hydrolyse tannic acid to gallic acid and subsequently to pyrogallol. The previous reports could illustrate the removal of inhibitory effect of tannic acid on N disappearance at time 24 and 48h. The values of N disappearance were 0.93, 0.91, 0.91 and 0.91 for 24h of incubation and 0.93, 0.94, 0.93 and 0.92 for 48h of incubation in T0, T1.5, T3.0 and T4.5, respectively.

Therefore, the reduction of N disappearance in treated alfalfa hay in the current study appears to be due to forming indigestible complexes with protein which leads to protect alfalfa protein from microbial degradation up to 12h of incubation and thus reducing the degradation rate (*c*) of protein.

In the present study the NDF in alfalfa hay presented about 40% of OM. Further effect of tannins was reported by McSweeney *et al.*, (2001) that tannins could reduce fiber digestion by complexing with lignocellulose and preventing microbial digestion or by directly inhibiting cellulolytic microorganisms or both. Therefore, the lower disappearance of OM could be attributed to effect of TA in the fiber digestion. The inverse correlation between the level of TA and *c* of OM in treated hay is in agreement with Makkar *et al.*, (1997). They also reported that addition of TA to hay reduced the production of short chain fatty acids. Reduction of *c* of OM would reduce the microbial protein synthesis as a result of lower energy supply in the form of fatty acid.

The results of the present study, would lead to the conclusion that TA decreased the solubilized N at time 0 and increased the potentially degradable fraction of protein, which could be beneficial to animal as it would decrease the excess of N in the rumen after feeding and contribute to maintain a more uniform availability of nitrogen over time. However, TA at levels of T3.0 and T4.5 may be inhibitory for VFA production as a subsequent effect for low degradation rate of OM.

Therefore, the level of TA at 1.5% of DM might achieve the objective of this study by enhancing the amount and extent of ruminal degradable fraction (*b*) of protein, meanwhile, this level of TA had insignificant effect on releasing of energy from OM.

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REFERENCES

A.O.A.C. (1990). Official Methods of Analyses. Vol. 1. 15th ed. AOAC, Arlington, VA.

Barry, T.N. and T.R. Manley (1984). The role of condensed tannins in the nutritional value of *Lotus pendaculatus* for sheep. Part 2. Quantitative digestion of carbohydrates and proteins. Br. J. Nutr., 51:493

Driedger, A. and E.E. Hatfield (1972). Influence of tannins on the nutritive value of soybean meal for ruminants. J. Anim. Sci.; 34: 465.

El-Waziry, A.M.; H.M.E. Kamel and M.H.M. Yacout (2000). Effect of backers' yeast (*Sacccharomyces cerevisiae*) supplementation to berseem (*Trifolium alexandrinum*) hay diet on protein digestion and rumen fermentation on sheep. Egyptian J. Nutrition and Feeds, 3: 71.

Goering, H.K. and P.J. Van Soest (1970). Forage fiber analysis (Apparatus, reagent and some application) Agric. Handbook no. 379. Agric. Res. Serv. USDA, Washington, DC.

Hoover, W.H. and S.R. Stockes (1991). Balancing carbohydrates and proteins for optimum rumen microbial yeild. J. Dairy Sci., 74: 3630.

Huntington G., B. (1989).Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a concentrate diet. Can. J. Anim. Sci., 69: 215.

Kamel, H.E.M., A.M. El-Waziry and J. Sekine (2000). Effect of *Saccharomyces cerevisiae* on fiber digestion and ruminal fermentation in sheep fed Barseem hay (*Trifolium alexandrinum*) as a sole diet. Proceedings of the 9th Congress of Asian-Australasian Association of Animal Production Societies and 23rd Biennial Conference of the Australian Society of Animal Production. Sydney, Australia. Vol.13: 139.

Kamel, H.E.M.; J. Sekine; T. Suga and Z. Morita (1995a). The effect of frozen-rethawing technique on detaching firmly associated bacteria from *in situ* hay residues. Can. J. Anim. Sci.; 75:481.

Kamel, H.E.M.; J. Sekine; T. Suga and Z. Morita (1995b). Effect of kinds of hay given to sheep on rumen degradability of protein of various hays. Anim. Sci. Technol. (Jpn.), 66: 36.

Kamel, H.E.M.; J. Sekine; T. Suga and Z. Morita (1995c). Degradations of dietary nutrients and purine in the rumen of sheep given oats, timothy and alfalfa hays. Anim. Sci. Techonl. (Jpn), 66:927.

Makkar, H.P.S.; M. Blummel and K. Becker (1997). Application of an *in vitro* gas method to understand the effect of natural plant products on availability and partitioning of nutrients. In: *In Vitro* technique for measuring nutrient supply to ruminants. Proceedings of Occasional Meeting of the British Society of Animal Production, University of Reading, UK.

McDonald, I (1981). A revised model for the estimation of protein degradation in the rumen. J. Agric. Sci., (Camb.), 96:251.

McSweeney, C.S.; B. Palmer, D.M. McNeil and D.O. Krause (2001). Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci. Technol. 30: 21.

Meherz, A.Z. (1992). Influence of roughage : concentrate ratio on N requirements of rumen microbes for maximal rate of fermentation. Proceedings of the International Conference on Manipulation of Rumen Microorganisms to Improve Efficiency of Fermentation and Ruminal Production. Alexandria, Egypt. 234.

Meherz, A.Z. and E.R. Ørskov (1977). A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. (Camb.), 88: 645.

Odenyo, A.A. and P.O. Osuji (1998). Tannin-tolerant ruminal bacteria from East Africa ruminants. Can. J. Microbiol., 44: 905.

Ørskov, E.R. and I. McDonald (1979). The estimation of protein degradability in the rumen from incubation measurements weighed according to the rate of passage. J. Agric. Sci. (Camb.), 92: 499.

SAS, I., Inc. (1994) SAS User's Guide: Statistics. Version 2 edition. SAS Institute, Inc., Cary, NC.

Satter, L.D. and L.L. Slyter (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. Brit. J. Nutr. 32: 199.

Sinclair, L.A., P.C. Garnsworthy, J.R. Newbold and P.J. Buttery (1995). Effect of sychronizing the rate of dietary energy and nitrogen release in diets with similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. (Camb.), 124:463.

Singh, B.; T.K. Bhat and O.P. Sharma (2001). Biodegradation of tannic acid in an *in vitro* ruminal system. Livestock Pro. Sci., 68: 259.

تأثير حامض التانيك علي تحلل البروتين والمادة العضوية لدريس البرسيم الحجازي

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أجريت هذه الدراسة للتعرف علي تأثير مستويات مختلفة من حامض التانيك علي تحلل البروتين و المادة العضوية في دريس البرسيم الحجازي باستخدام طريقة ال *in situ* في الأغنام المغذاه علي دريس الشوفان في تصميم مربع لاتيني.

تم معاملة دريس البرسيم الحجازي بمستويات مختلفة من حامض التانيك وهي صفر (صفر T)& ١,٥(١,٥ T) & ٣ (٣ T)& ٤,٥ (٤,٥ T)% من المادة الجافة.

التأثير المثبط لحامض التانيك علي اختفاء النتروجين كان معنويا ($P<0.05$) الي ١٢ ساعة من التحضين في المعاملة ٤,٥ T و التأثير أستمر حتى ٦ ساعات في المعاملة ١,٥ T و ٣,٠T.

المستوي العالي من التانيك كان له تأثير معنوي علي خفض المادة العضوية المتحللة عند أزمنة التحضين ٦و ١٢ ساعة. كما أن المادة العضوية المتحللة عند زمن ٢٤ و ٤٨ ساعة لم تتأثر بالمستويات المستخدمة من التانيك.

كمية البروتين البطيء التحلل في الكرش ذادت معنويا ($P<0.05$) في الدريس المعامل بحامض التانيك. كما أنة وجدت علاقة عكسية بين سرعة تحلل البروتين في الكرش و مستوي التانيك.

كمية المادة العضوية السريعة والبطيئة التحلل في الكرش لم تتأثر معنويا بحامض التانيك بينما انخفضت سرعة تحلل المادة العضوية معنويا ($P<0.05$) في المعاملة ٣ T و ٤,٥T.

أوضحت نتائج هذه الدراسة أن المستوي المنخفض من حامض التانيك (١,٥ T) أدي الي انخفاض مرغوب لتحلل البروتين بينما تأثيرة علي تحلل المادة العضوية لم يكن معنويا.