Journal of Animal and Veterinary Advances 11 (17): 3094-3099, 2012
ISSN: 1680-5593

Effect of Additives on the Quality and *in vitro* Digestibility of Alfalfa Hay with High Moisture Content

Huajia Shan, Fuyu Yang, Wenxu Zhang, Ligang Qin and He Zhou
Department of Grassland Science, College of Animal Science and Technology,
China Agricultural University, 100193 Beijing, China

Abstract: Two experiments were conducted to investigate the problems of severe nutrient loss of traditional alfalfa hay processing methods and storage difficulties in moist or rainy conditions. Alfalfa hay baled at two percentages of moisture (18 and 27%), respectively were taken as controls (CKL, low-moisture control; CKH, high-moisture control). Bale treatments were sampled before storage and at 5, 10, 25 and 60 days postbaling. Nutrient content and the curve of nutrient change during 60 days storage were analyzed to study the effect of compound additives on the quality and organic-matter digestibility of alfalfa hay baled at high-moisture content. The results showed that the nutrient-component proportion of conventional low-moisture alfalfa hay changed inconspicuously during the 60 days storage process and high-moisture treatment combinations that favored extended microbial activity continued to decrease the forage quality (particularly, increased neutral-detergent fiber and acid-detergent insoluble nitrogen). Nutrient content and *in vitro* digestibility of high-moisture alfalfa hay processed with compound additives improved to varying degrees after 60 days preservation. Its water content was 1.62% points less than that of the high-moisture control (CKH). Crude protein and soluble carbohydrates increased by 0.42 and 6.75 g kg^{-1}, respectively while non-protein nitrogen and crude fiber decreased by 0.39 and 3.23% g kg^{-1}. At the same time, *in vitro* dry matter digestibility and *in vitro* crude protein digestibility greatly increased by 4.18 and 2.74%.

Key words: Alfalfa hay, compound additives, high-moisture content, *in vitro* digestibility, China

INTRODUCTION

Alfalfa hay is one of the best forages in the modern animal husbandry industry. However, loss from dry matter and quality during harvest and storage are very great in most countries (Knapp *et al.*, 1975; Rotz and Abrams, 1988). Typical dry matter losses for the full process are 15-25% (Table 1) for hay made under good drying conditions and 35-100% for hay damaged by rain (Harris *et al.*, 1974; Brandt *et al.*, 1984; Lines *et al.*, 1996; Hundtoft, 1965). Alfalfa leaves which are highest in nutritive value to livestock are most susceptible to loss resulting in an overall reduction of crop quality. Leaves of alfalfa hay that contain low moisture fall off in great numbers during the process of field curing, baling, transportation and storage (Johnson *et al.*, 1984; Wilkinson, 1981; Collins *et al.*, 1983).

Baling alfalfa hay at moisture concentrations above 200 g kg^{-1} can cause negative quality changes via spontaneous heating within the hay mass, Maillard reactions and increased concentrations of field and storage fungi (Robert, 1995). Spontaneous heating and associated loss of energy occur due to respiration by both plant enzymes and a variety of microorganisms associated with the hay. In moist hays (>200 g kg^{-1}), microbial respiration, primarily via oxidation of nonstructural carbohydrates can cause substantially elevated temperatures (>50°C) in the hay mass (Rotz and Muck, 1994). Microbial populations are sensitive to both temperature and moisture content substantial changes in these populations are known to occur in response to temperature changes and the dissipation of water from the hay (Robert *et al.*, 1987). This not only causes more serious nutrition loss but also

Table 1: Typical losses of forage-handling systems

Moisture content when mowed	Field loss (%)	Storage loss (%)	Total loss (%)
Heat-cured dry	12-18	3-4	15-22
Field-cured dry (40% moisture)	12-18	4-6	16-24
Field-cured dry (20% moisture)	15-22	2-4	17-26

Hundtoft (1965)

Corresponding Author: He Zhou, Department of Grassland Science, College of Animal Science and Technology, China Agricultural University, 100193 Beijing, China

reduces digestibility and palatability significantly (Brandt *et al*., 1984). Typically, these complex processes gradually reduce the moisture content of the hay to <180 g kg^{-1} after a month in storage, thereby limiting microbial activity and establishing relatively stable conditions in the hay mass (Rotz and Muck, 1994).

Alfalfa hay treated with additives and baled at a high-moisture level could tolerate the severe losses caused by both serious falling off of leaves and mildewing. Especially during the rainy season in moist areas, additives, a useful tool to use to avoid a great loss of nutrition have great practical significance for efficiency and quality improvement in forage processing and animal breeding.

MATERIALS AND METHODS

Experimental site: The experiments were conducted during 2008-2009 at Beijing Dairy Cattle Centre and China Agriculture University. To estimate the nutritive value and quality of alfalfa hay, its chemical composition and corresponding digestibility coefficients were used. Studies included nutritional change of alfalfa hay treated with additives during 60 days storage (Trial 1) and the effect of additives on i*n vitro* digestibility (Trial 2). The alfalfa used in the experiment was third-cut Golden Empress alfalfa planted at the YanQing Farm (Beijing) in 2007. It was mainly in the pod-developmental stage when harvested. Experimental animals were three lactating surgically modified Holstein cows fed a total mixed ration composed of corn silage and alfalfa hay at the College of Animal Science of China Agriculture University.

Experimental procedure

Trial 1-nutrition change: Additives include Silo Guardll (SG, 2.5 kg ton^{-1}) and a new compound additive (CA, 2.9 kg ton^{-1}) designed and developed by the Department of Grassland Science of China Agricultural University. All additives were diluted with distilled water (1:1) and sprayed evenly on the alfalfa immediately after cutting. Alfalfa hay was packaged at the moisture concentrations of 27%. Treatments were replicated three times in this experiment. During the 60 days storage process, triplicate samples were collected respectively on 0, 5, 10, 25 and 60 days. Two different batches of alfalfa hay without additives with a water content of 27 and 18%, respectively were used for control treatments (CKH and CKL).

Trial 2-external digestion experiment: Samples of alfalfa hay with 0 day storage (CKH0, low-moisture control without storage CKH0, high-moisture control without storage) with a water content of 27 and 18% were used for control treatments in this experiment in order to compare the *in vitro* digestibility of the different treatments (SG, CA, CKH and CKL). *In vitro* digestibility of alfalfa forage was estimated by two-stage methods in which the various samples were kept completely separate from one another using dialysis bags (Wilman and Adesogan, 2000). The methods were based on those recommended by ANKOM Technology (ANKOM Technology, Fairport, NY, USA) using 0.5 g of sample per bag and 3 bags per incubation tube (100 mL capacity). The ruminal fluid was collected 2 h after the morning feeding from three surgically modified cows.

Table 2: Confecting Method of Buffer Solution for *in vitro* incubating

Reagent	Chemical formula	Concentration (g L^{-1})
Sodium bicarbonate	$NaHCO_3$	9.80
Disodium hydrogen phosphate	$Na_2HPO_4{\cdot}H_2O$	9.30
Sodium chloride	NaCl	0.47
Potassium chloride	KCl	0.57
Urea	$CO(NH_2)_2$	1.00
Calcium chloride	$CaCl_2$, anhyd	0.04
Magnesium chloride	$MgCl_2$, anhyd	0.06

Van Soest (1994)

The first step in simulating the digestion in the rumen was fermentation in the mixture (1:1) of ruminal fluid and buffer solution (Table 2) which had been warmed to 39°C in an incubator and flushed with oxygen-free CO_2 for 48 h. The second step was hydrolysis with pepsin solution (0.2%, 0.2 g pepsin dissolved in a liter 1 mol L^{-1} hydrochloric acid) at 39°C for 48 h.

Chemical analyses: After drying and grinding, forage samples were analyzed for Dry Matter (DM), Crude Protein (CP), Non-Protein Nitrogen (NPN), Water-Soluble Carbohydrate (WSC), Neutral-Detergent Fiber (NDF), Acid-Detergent Fiber (ADF), *In Vitro* Dry-Matter Digestibility (IVDMD), *In Vitro* Neutral-Detergent Fiber Digestibility (IVNDFD), *In Vitro* Acid Detergent-Fiber Digestibility (IVADFD) and *In Vitro* Crude-Protein Digestibility (IVCPD). The amount of CP was determined using the DigiPREP TKN Systems (BUCHI 339). The alfalfa hay was analyzed for DM (method 930.15) and NPN (method 990.03) according to AOAC in 1995. Extraction of WSC was carried out according to the method described by Terashita *et al.* (1992). The content of WSC in alfalfa hay was identified by anthrone-sulfuric acid colorimetry with wavelength at 624±1 nm (Harada *et al*., 2003). The NDF and ADF, both inclusive of residual ash were determined according to Van Soest *et al.* (1991) and Robertson, respectively with the methods modified for use with an ANKOM^{200} fiber analyzer (ANKOM Technology).

Statistical analyses: The linear relationships between nutrition content and storage were analyzed using the

Statistical Product and Service Solutions (SPSS) statistical package (Version 13.0 for Windows). All data in this study were tested at significance levels of $p<0.05$ and $p<0.01$ by one-way ANOVA. Differences among treatment means were tested with the Duncan multiple range test considered significant if the $p\leq 0.05$ and 0.01.

RESULTS

Change of moisture and nutrition: The moisture content of SG and CA decreased to 19.39 and 18.79%, respectively, in the 1st 10 days immediately after baling (Fig. 1 and 2). Differing from the additive treatment results the moisture content of CKH and CKL decreased slowly both at the very beginning and at the latter part of the storage process. Finally, the moisture content of SG and CA had decreased to 12.02 and 11.91%, very close to CKL's moisture (11.72%) after 60 days of storage. There was no discernible difference in corresponding moisture-reducing coefficients between SG and CA.

The CP content of the CKL was the lowest of all treatments because of severe losses during the process of field curing, baling, transportation and the 60 days storage process (Fig. 3). Differing from the steadily lowered CP content of CKL, the CKH reduced dramatically from 19.76-18.04% in the 1st 20 days for the losses caused by spontaneous heating and microbial multiplying. CP contents of alfalfa hay processed with additives were obviously higher than others at the end of the storage process. The CA was slightly higher (0.09%) than that of the SG.

The NPN content of CKL did not change obviously during the entire storage process (Fig. 4). The NPN content of CKH had two obvious fluctuations because of spontaneous heating and microbial multiplying. It increased significantly and achieved peak maximum in the 1st 10 days. The minimum value appeared on day 20 and dropped to 4.97 g kg^{-1}. After the first fluctuation, it rose again until the NPN content reached 5.6 g kg^{-1} around day 30. The decline of SG and CA was very apparent in the latter part of the storage process.

The changes of fiber content are shown in Fig. 5. The CKL fiber content was obviously higher than that of other treatments at the very beginning of the storage period because the stems made up a growing percentage given the falling off of leaves. This was a rapidly increasing process of fiber content for the CKH because of the

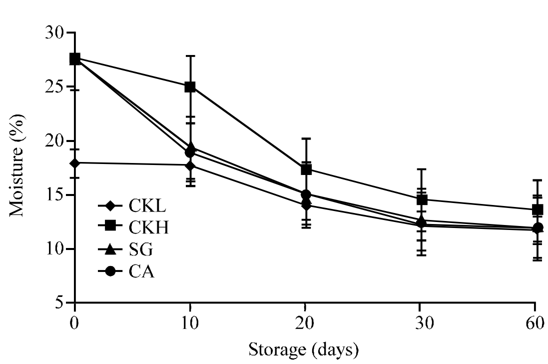

Fig. 1: Change of moisture content of different treatments during 60 days storage process

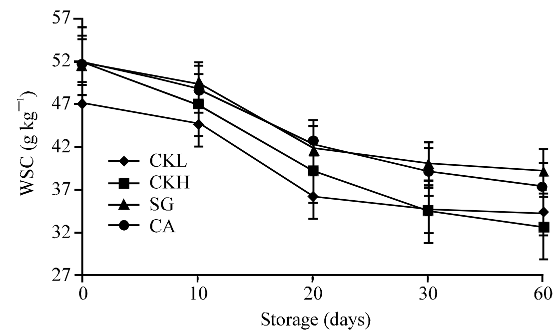

Fig. 2: Change of WSC content of deferent treatments during 60 days storage process

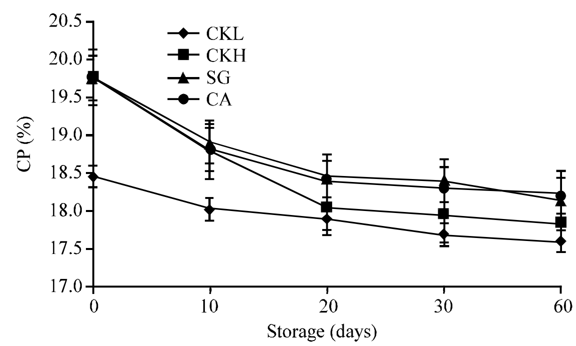

Fig. 3: Change of CP content of different treatments during 60 days storage process

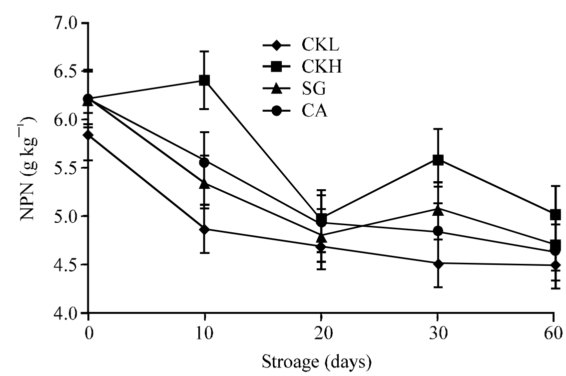

Fig. 4: Change of NAN content of different treatments during 60 days storage process

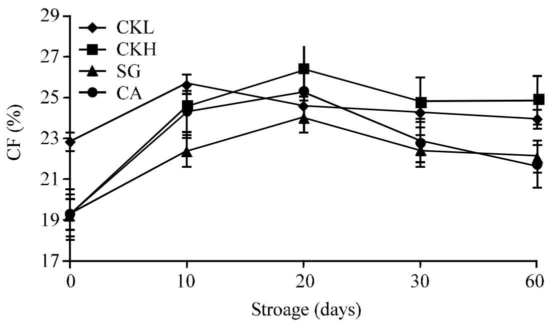

Fig. 5: Change of CF content of deferent treatments during 60 days storage process

Table 3: Nutrient contents of alfalfa hay with different treatments after 60 days storage process

	CP	NPN	WSC	NDF	ADF
Contents	----------------	----------------	(g kg^{-1} DM)	----------------	----------------
CKL	175.8^{cC}	4.49^{cC}	34.48^{bB}	165.9^{cC}	240.1^{bB}
CKH	178.2^{bB}	5.02^{aA}	32.67^{bB}	184.2^{aA}	249.4^{aA}
SG	181.5^{aA}	4.71^{bB}	39.42^{aA}	172.1^{bB}	221.8^{cC}
CA	182.4^{aA}	4.62^{bBC}	37.63^{aA}	174.8^{bB}	217.1^{cC}

$^{a-c}$Values in the same column with differences are significantly different at $p<0.05$. $^{A-C}$Values in the same column with differences are significantly different at $p<0.01$

severe nutrient oxidation and degradation. Finally, the percentage of fiber reached 24.94% after 60 days in storage. Fiber contents of SG and CA had increased in a small way.

Nutrient contents at the end of the 60 days storage period of different treatments are shown in Table 3. The CP and NPN contents of CKL were the lowest of all because of the serious loss caused by leaves falling off. Nutrients of CKH were distinctly reduced by spontaneous heating and microbial multiplying. The CP and WSC were decomposed by a variety of microorganisms' associated respiration and the percentages of NPN fiber improved obviously. Nutrients of alfalfa hay processed with additives were much higher than those of the control treatments and the difference was very significant.

***In vitro* digestibility analysis:** The IVDMD of different treatments are shown in Fig. 6. The IVDMD of CKH0 and CKL0 was distinctly higher than treatments that had 60 days of storage. The CA DM digestibility (62.64%) was slightly higher than the SG (62.29%) but the difference was not significant.

The IVNDFD and IVADFD of the CKL0 (58.12 and 48.69%) shown in Fig. 7 and 8 were much higher than that of other treatments. The IVNDFD and IVADFD of the CKH0 (34.49 and 35.65%) were the lowest of all. Compared with alfalfa hay processed with additives, the IVNDFD and IVADFD of CKL (53.81 and 43.14%) were obviously higher than other treatments stored for 60 days.

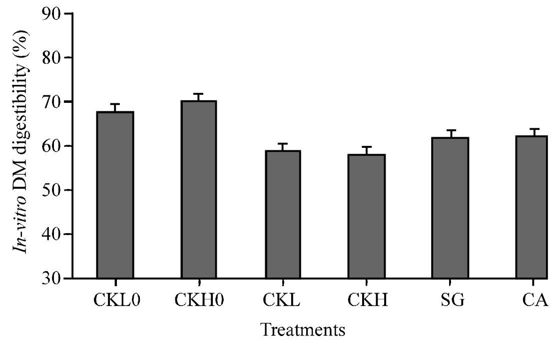

Fig. 6: *In vitro* DM digestibility of different treatments

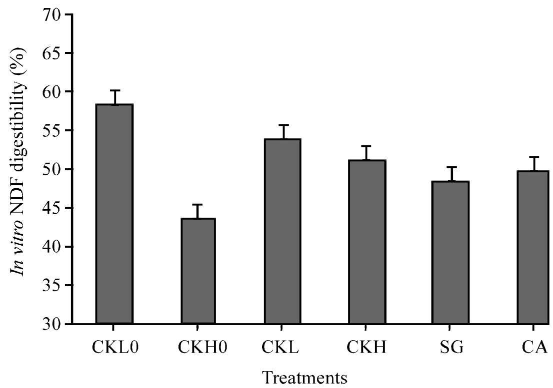

Fig. 7: *In vitro* NDF digestibility of different treatments

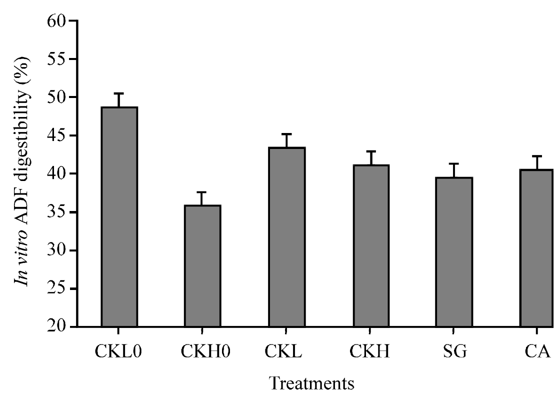

Fig. 8: *In vitro* ADF digestibility of different treatments

The IVCPD of CKH0 (91.45%) was distinctly the highest of that of all treatments (Fig. 9). Compared with CKH0, CP digestibility of CKL0 (83.17%) was very close to the levels of the SG and CA. The IVCPD of CA (84.97%) was higher than that of others in the storage-treatment groups and the difference was very significant.

In vitro digestibility of different treatments was shown in Table 4. The DM and CP digestibility of CKL0 was very low while the IVNDFD and IVADFD were higher

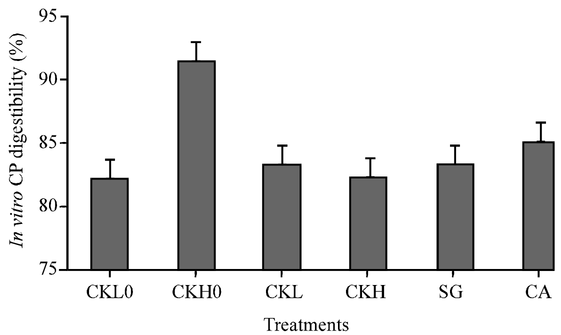

Fig. 9: *In vitro* CP digestibility of different treatments

Table 4: *In vitro* digestibility of different treatments

Treatments	IVDDM (%)	IVNDFD (%)	IVADFD (%)	IVCPD (%)
CKL0	68.26^{bB}	58.12^{aA}	48.69^{aA}	83.17^{cC}
CKH0	70.71^{aA}	43.49^{eE}	35.65^{eD}	91.45^{aA}
CKL	59.17^{dD}	53.81^{bB}	43.14^{bB}	82.31^{dC}
CKH	58.46^{dD}	50.84^{cC}	40.81^{cC}	82.23^{eC}
SG	62.29^{cC}	48.39^{dD}	39.32^{dC}	83.28^{cC}
CA	62.64^{cC}	49.71^{cdCD}	40.36^{cC}	84.97^{bB}

$^{a-e}$Values in the same column with differences are significantly different at $p<0.05$. $^{A-D}$Values in the same column with differences are significantly different at $p<0.01$. IVDMD: *In Vitro* Dry Matter Digestibility; IVNDFD: *In Vitro* Neutral Detergent Fiber Digestibility; IVADFD: *In Vitro* Acid Detergent Fiber Digestibility; IVCPD: *In Vitro* Crude Protein Digestibility

than that of other treatments. The reason may be that nutrient losses were severe during the course of the alfalfa hay processing and percentages of CP and digestible nutrients declined. Compared with the CKL0, the nutrient digestibility of the CKL changed insignificantly. This means that nutrients losses of low-moisture alfalfa hay were slight during the 60 days storage process. The IVDMD and IVCPD of CKH0 were obviously higher than that of other treatments. Nevertheless *in vitro* digestibility of high-moisture alfalfa hay decreased rapidly with the severe nutrient loss caused by the spontaneous heating and microbial multiplying during the 60 days of storage. The IVDMD and IVCPD of alfalfa hay processed with additives were obviously higher compared with that of the CKL and CKH. It can be deduced that additives can effectively thwart nutrient loss during alfalfa hay processing.

DISCUSSION

This experiment shows that the nutrient-component portion of alfalfa hay with low-moisture content changed inconspicuously in the 60 days storage process. The digestibility of the CKL and CKL0 was lower than that of other treatments because of the low-nutrient content. Although, the nutrient content of the CKH was higher than the CKL at the very beginning of the experiment it decreased rapidly during the 60 days storage period with the spontaneous heating, microbial multiplying and respiration of forage grass. Digestibility changes of alfalfa hay with high-moisture content in the preservation process were the most significant of all. Nutrient contents of alfalfa hay baled at a high-moisture content were significantly higher than that of hay baled at a low-moisture content. Further, the nutrient consumption of alfalfa hay treated with compound additives was obviously less than that of the CKH during the storage process. At the same time, *in vitro* digestibility of alfalfa hay processed with compound additives improved significantly. The results prove that compound additives can address the contradiction of nutrient loss and mustiness caused by different moisture contents. Compared with the international brand (the additive Silo Guardll), the CP content of the CA was slightly higher while the NPN and fiber content were lower.

Two of the most important reproductive conditions of microbials are appropriate temperature and moisture content. Moisture is an essential factor of growth and reproduction of mold. High-moisture treatment combinations that favor extended microbial activity continued to depress forage quality. Analysis results showed that compound additives (CA and SG) can rapidly reduce the moisture content of alfalfa hay during the storage stage immediately after baling. Some ingredients of compound additives (Na_2SO_3) consumed oxygen in the process of oxidation. Decrease of moisture content and consumption of oxygen broke the survival environment for the microbials and reduced the rot probability of the alfalfa hay. These effects can also reduce the losses caused by respiration of forage grass. All evidence suggests that compound additives had positive effects on reducing severe losses during the processing link and reducing mildewing in the storage process. Thus, CA as a new compound additive has the same effect as the international brand Silo Guardll.

The nutrition of forage grass has vital significance for livestock. It provides energy, participates in the body's metabolism and is even involved in the composition of meat, milk, skin, hair, bone and other tissue organs directly. This means that the quality and economic value of alfalfa hay to a great extent depends on different proportions of nutrients (Wittenberg and Moshtaghi-Nia, 1990). A great deal of experiments and research shows that the levels of moisture content and processing technology have a very serious influence on the nutrient contents of alfalfa hay. It is an effective method to reduce the severe nutrient loss caused by leaves falling off and the mildewing processes of alfalfa hay baled at high-moisture content with compound additives. It also could achieve the purpose of improving the quality and economic efficiency of alfalfa hay via increasing the proportion of protein and other nutrients.

CONCLUSION

Experiments show that this is an effective method to reduce the severe nutrient loss caused by leaves dropping and mildewing processing alfalfa hay baled at high-moisture content with compound additives.

REFERENCES

Brandt, B.M., T. Nelson, B. Klopfenstein and B. Britton, 1984. Influence of silage inoculant on the yield and preservation of alfalfa baled at three moisture levels. Nebraska Beef Cattle Report.

Collins, M., 1983. Wetting and maturity effects on the yield and quality of legume hay. Agron. J., 75: 523-527.

Harada, A, S. Yoneyama, S.C. Doi and M. Aoyama, 2003. Change in contents of free amino acids and soluble carbohydrates during fruit-body development of *Hupsizygus marmoreus*. Food Chem., 83: 343-347.

Harris, C. E., R. Thaine and H.I.M, Sarisalo, 1974. Effectiveness of some mechanical, thermal and chemical laboratory treatment on the drying rate of leaves and stem internodes of grass. J. Agric. Sci., 83: 353-558.

Hundtoft, E.B., 1965. Extension and research cooperate evaluating forage harvesting systems. Am. Soc. Agic. Eng., 24: 635-635.

Johnson, D.G., D.E. Otterby, R.G. Lundquist, J.A. True and F.A. Benson *et al*., 1984. Yield and quality of alfalfa as affected by harvesting and storage methods. J. Dairy Sci., 67: 2475-2480.

Knapp, W.R., D.A. Holt and V.L. Lechtenberg, 1975. Hay preservation and quality improvement by anhydrous ammonia treatment. Agron. J., 67: 766-769.

Lines, L.W., M.E. Koch and W.P. Weiss, 1996. Effect of ammoniation on the chemical composition of alfalfa hay baled with varying concentrations of moisture. J. Dairy Sci., 79: 2000-2004.

Robert, C.A., 1995. Microbiology of Stored Forages. In: Post-Harvest Physiology and Preservation of Forages, Moore, K.J. and M.A. Peterson (Eds.). American Society of Agronomy and Science Society America, Madison, WI., USA., pp: 21-38.

Robert, C.A., K.J. Moore, D.W. Graffis, H.W. Kirby and R.P. Walgenbach, 1987. Chitin as an estimate of mold in hay. Crop Sci., 27: 783-785.

Rotz, C.A. and R.E. Muck, 1994. Changes in Forage Quality During Harvest and Storage. In: Forage Quality, Evaluation and Utilization, Fahey, G.C. (Ed.). ASA, CSSA, SSSA., Madison, WI, USA., pp: 828-868.

Rotz, C.A. and S.M. Abrams, 1988. Losses and quality change during alfalfa hay harvest and storage. Am. Soc. Agric. Biol. Eng., 31: 350-355.

Terashita, T., M. Kono, J. Shishiyama and M. Yamauchi, 1992. Constituents in fruit-bodies of *Lentinus edodes* singer grown on sawdust rice bran beds (artificial bed-blocks). The proximate components, free and protein-bound amino acids in protein and 5'-GMP. Rep. Environ. Sci. Res. Inst. Kinki Univ., 20: 117-124.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. 2nd Edn., Cornell University, Ithaca, NY., USA.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Wilkinson, J.M., 1981. Losses in the conservation and utilization of grass and forage crops. Ann. Applied. Biol., 98: 365-375.

Wilman, D. and A. Adesogan, 2000. A comparison of filter bag methods with conventional tube methods of determining the *in vitro* digestibility of forages. Anim. Feed Sci. Technol., 84: 33-47.

Wittenberg, K.M. and S.A. Moshtaghi-Nia, 1990. Influence of anhydrous ammonia and bacterial preparations on alfalfa forage baled at various moisture levels. Nutrient composition and utilization. Anim. Feed Sci. Technol., 28: 333-344.