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Original Article

The Determination of Metabolizable Protein of treated Alfalfa with sunlight heat and formaldehyde

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ABSTRACT

The present study was carried out to determine the metabolizable protein (MP) of treated alfalfa, using nylon bags technique. Two fistulated whether with average BW 45±2 kg were used. The data was analyzed using completely randomized design. The experimental treatments were treatments A: control treatment, B: alfalfa treated with 0.4% formaldehyde and chopped before drying sun, C: alfalfa treated with 0.4% formaldehyde and D: alfalfa chopped before drying sun. The incubation times were 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h. The degradability parameters of crud protein (CP) for soluble fractions (a) were 8.16, 5.7, 7.15 and 5.88% and fermentable fractions (b) were 72.023, 37.113, 49.11 and 54.35% for treatments of A, B, C and D, respectively. The MP of treatments A, B, C and D were obtained 119.72, 131.39, 132.49 and 127.45 gkg^{-1}DM, showing a significant difference between four treatments. The alfalfa treated with 0.4% formaldehyde had high MP compared to others. These results showed that the processing of alfalfa with formaldehyde and sunlight heat caused high MP.

Keywords: Alfalfa, Formaldehyde, Metabolizable protein, Nylon bags, Sunlight heat.

INTRODUCTION

Metabolizable protein system was suggested by Miler in 1973. This system is based on microbial protein and undegradable digestible protein that can be digested and absorbed in the gastrointestinal tract after rumen. In this system, microbial protein entering the small intestine is calculated on the basis of effective rumen degradable protein. Effective rumen degradable protein consists of two fractions: quickly degradable protein (with efficiency 80%) and slowly degradable protein (Taghizadeh and Farhomand, 2007). The system will also determine the composition of protein absorption in small intestine as well as the real substance of dietary animal protein. This system is characterized by amino acids that can reach the actual consumption of animal metabolism.

Alfalfa is considered one of the most important forage and widely cultivated in subtropical regions. The original home of the world is Iran, however, around 450 BC its seeds are transferred by commercial convoys to Europe and North America. Alfalfa is of particular importance in the forage plants, because in addition to efficiency of feed per unit area and the high quality feed for livestock, it has grown to strengthen and improve soil and also have a positive impact on the amount of products the next crop in the rotation (Khanjani and Kalafchi, 2004). Ferdinand and Jung (2005) believes that alfalfa is good quality forage, because is a high crude protein and have the high digestibility compared to many of the forages. It is possible to meet the nutritional requirements of animals with the balanced rations and forage forms a significant part of the common diet of ruminants. Among forages, alfalfa is important because good quality, palatability and having food reserves of minerals, protein and vitamins (Karimi, 2001). Many factors affect the quality of the forage such as the type of forage plants, the contents of the different parts of plants especially leaves, harvesting and climatic conditions and management. Plant growth at harvest is the most important factor in the forage quality (Fazaeli, 1993).

Processing proteins with formaldehyde is the most common method of protecting proteins. Mengen *et al*., (1980) results that spraying farm with Formaldehyde not effective to harvest fresh hay as Glutar-aldehyde, because Glutar-aldehyde reacts faster and no toxic effects on rumen microorganisms. When protein is soaked in formaldehyde enter into an environment with high acidity (abomasum), performed reactions been reversed and protein becomes degraded. Treated with aldehydes had the high sensitivity, for example, it was reported that increase the use of formaldehyde in soybean an amount greater than 2 g/kg DM, reduced nitrogen solubility in phosphate buffer from 24% to 5% and release of ammonia in the lab from 55 grams to 15 grams of nitrogen per 100 g of placed nitrogen. The amount of formaldehyde is used depends on the amount of rumen degradable true protein and to factors such as carbohydrates, moisture level and particle size (Yang and Broderick, 1993).

MATERIALS AND METHODS

Alfalfa collection and process

Alfalfa was obtained from local variety of department of agriculture of Miandowab, Iran. The experimental treatments were treatments A: control treatment, B: alfalfa treated with 0.4% formaldehyde and chopped before drying sun, C: alfalfa treated with 0.4% formaldehyde and D: alfalfa chopped before drying sun.

Besides, 3 parts of solution and 1 part of barley were mixed in plastic containers and were kept in room temperature and away from sunlight for 60 days; samples were taken out of the containers and dried in the sunlight and milled in a 2 mm size to be used in other phases of the experiment.

Animals used in this experiment were fed at maintenance level. The animals were fed with a mixture of 60% forage and 40% concentrate diet (Ørskov and McDonald, 1979).

Chemical Composition

Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30), and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (1990). The neutral detergent insoluble fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using the methods of Van Soest *et al*. (1991), without sodium sulphite. Neutral detergent insoluble fiber was analyzed without amylase with ash included.

Measured in Situ Method

To estimate the degradability of the nylon bag technique, the food samples were milled with a special mill and 2-mm sieve (Moghaddam *et al*., 2012). 5 grams of each nutrient were

poured into bags made of synthetic polyester fiber as 6 × 12 cm and pore diameter of 50 mm. Two fistulated sheep with average BW 45 ± 2.5 kg were used in a complete randomized design to determine the degradation at time zero, sample bags were washed under tap water for 15 minutes. Incubation times were 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h.

After each incubation time, the bags were removed and rinsed with cold water until the water is completely cleared out. After washing, bags were incubated for 24 h at a temperature of 65˚C to evaporate and for 24 h at 105˚C in oven (Moghaddam *et al*., 2012).

Degradation parameters (soluble, insoluble, and fixed rate of degradation) were calculated with Naway. For matched degradation data used from $P = a + b (1 - e^{-ct})$ that a= The degradation of soluble fraction (%), b= The degradation rate of insoluble fraction (%), c= The constant degradation rate (%/h), t=The incubation time (h), e= The constant factor (2.718) and P= The degradation rate at the time t. Effective degradability was calculated at $ED = [a + (b \times c)] \div (c + k)$ that k is passage rate which were considered in this study 0.02.

Statistical Analysis

The obtained data from in situ study was analyzed according to a completely randomized design with 4 replicates by the GLM procedure (SAS, 2002). The treatment means were compared by the Duncan test.

RESULTS AND DISCUSSION

Ruminants, differently from monogastric animals used in feed proteins that portion of the feed protein is degraded by microorganisms in the rumen and spend of the microbial protein. The use of protein degradability characteristics of feed used as a means of expression in the new system of the feed protein value (Moghaddam, 2010).

The subjects in this experiment, the metabolizable protein treatment C (132.49 g/kg DM) and treatment A (119.72 g/kg DM) accounted for the highest and lowest values and these were statistically significantly different (P<0.05). The difference in protein metabolism, among treatments can be attributed to the chemical composition, variety of different proteins especially buffer insoluble proteins and acid detergent insoluble protein, so difference in crude protein and degradation characteristics of the material that leading to a decrease in metabolizable protein in control treatment.

Effective ruminal degradable protein represents the total amount of nitrogen in the rumen that the rumen microorganisms consume for their growth. The more the level of food intake increases, the more effective ruminal degradable protein value decreases due to the increase in the rate of passage of food from the rumen (Moghaddam, 2010).

As it is shown in Table 2, there was a significant difference among the effective ruminal degradable protein of treatments (P<0.05). The difference effective ruminal degradable protein of treatments, on the one hand is the difference in the percentage of protein samples and the water-soluble (a) amount which is the difference of activity of rumen microorganisms and differences in crude protein degradability.

Table 1: Metabolizable protein components of treatments (g/kg DM)

Treatments	QDP	SDP	ERDP	UDP	DUP	MP
A	14.31^{a}	125.9^{a}	137.35^{a}	35.48^{d}	31.82^{d}	119.72^{c}
B	10.01^{c}	65.11^{d}	73.12^{d}	100.57^{a}	90.36^{a}	131.39^{a}
C	12.72^{b}	87.09^{c}	97.27^{c}	78.19^{b}	70.24^{b}	132.49^{a}
D	10.17^{c}	93.82^{b}	101.96^{b}	69.24^{c}	62.19^{c}	127.45^{b}
SEM	0.291	2	0.95	1.8	1.62	0.69

QDP=quickly degradable protein, SDP=slowly degradable protein, ERDP=effective ruminal degradable protein, UDP=undegradable digestible protein, DUP=digestible undegradable protein, MP=metabolizable protein.
SEM= Standard error means of the difference amount three treatments means
a,b: Within a column, means without a common superscript letter differ (P< 0.05)

Means data of crude protein and crude protein degradability coefficients of treatments are listed in Tables 2. According to the results, treatment A (72.023%) and treatment B (37.113%) were the highest and lowest amount of coefficient (b) in the rumen. Alizald *et al.*, (1999), reported alfalfa degradation coefficients were (a = 40.7% and b = 50.6%), that are different with obtained data in this study which this difference can depend on the differences in the used varieties, sampling and the basal diet of animals studied, the size of the holes in plastic bags, microbial contamination, washing method sacs and the processing effect.

Since the DM is a mixture of crude protein, fat, carbohydrates, and vitamins and the tested feeds treated with formaldehyde were different with regard to these nutrients, therefore the reduced ruminal degradation of starch and crude protein is not due to the toxicity effects of formaldehyde on microorganisms in the rumen, but rather it is due to the methylene cross-linking proteins in the alfalfa field that reduced the sensitivity of microbial degradation of forage protein and microorganisms access to starch. Consequently, it increases the delay phase in rumen degradation of protein and starch. Also formaldehyde can be bonded with a protein and Inhibit invasion microorganisms to protein degradation (Ahmadi, 2013).

Drying the chopped alfalfa in the sun, increase of the (b) fraction of protein which is caused by the absorption of more sunlight during drying due to its darker color. Consequently, the increase in temperature and enhance the reaction of sugars with proteins (Yang and Broderick, 1993). Compared with non-shredded grass, chopped alfalfa had the lower ADF, regenerative sugar, ADIN, (a) fraction of protein and had the higher (b) fraction of protein. Of course, heat of the sun is useful when the reactions between sugars and proteins are reversible (Yang and Broderick, 1993).

Table 2: The parameters estimated from the crude protein degradation of treatments

Treatments	a	b	c	ED	CP
A	8.16^{a}	72.023^{a}	4.86^{b}	79.98^{a}	17.56^{a}
B	5.7^{c}	37.113^{d}	6.41^{a}	42.74^{d}	17.57^{a}
C	7.15^{b}	49.11^{c}	5.13^{b}	55.44^{c}	17.8^{a}
D	5.88^{c}	54.35^{b}	6^{a}	60.04^{b}	17.32^{a}
SEM	2.3	0.734	0.162	0.75	0.321

a=crude protein solution at time zero (%), b= crude protein fermentable (%), c=Constant analysis factor at time t (% per hour), ED=effective degradability (%) (r=0.02), CP=crude protein.
SEM= Standard error means of the difference amount three treatments means
a,b: Within a column, means without a common superscript letter differ (P< 0.05)

CONCLUSION

Given the obsolescence of formaldehyde in ruminant nutrition, this experiment proved that it can be use the heat of the sun instead of formaldehyde that increased metabolizable protein and do not have toxic and negative effects of formaldehyde. Drying in the sun is a thermal processing that leading to increase of metabolizable protein and with chopped hay before drying increased the surface is placed in the sun and increases the effect of sunlight.

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