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**EFFECT OF CHEMICAL COMPOSITION OF ALFALFA HAY ON SEVERAL
DIGESTIVE MEASUREMENTS IN GROWING RABBITS**

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Abstract

Seventy five New Zealand White x Californian rabbits were used to study the influence of chemical composition of alfalfa hay on caecal and caecotrophy characteristics in growing rabbits. Five alfalfa hays were selected varying from 387 to 550 g NDF/kg DM and 35.2 to 27.0 g N/kg DM. Caecum, caecal contents and whole digestive tract weight (% body weight) and caecal NDF increased linearly ($P=0.001$) with the NDF content of diet. This fact implied the impairment of carcass performance when increased the fibre of diet. A linear decrease with NDF content of diet was observed for caecal N ($P=0.001$) and ammonia concentration ($P=0.024$), the lowest value of ammonia concentration being 15.4 mg N-NH₃/100 ml. A quadratic tendency ($P=0.062$) of type of diet was observed for the total VFA content, the extreme diets having the highest concentrations (as average 87.9 mmol/l). Caecal pH (as average 5.76) and VFA molar proportions (as average 79.3, 6.6 and 14.1% for acetic, propionic and butyric acid respectively) did not vary with type of diet. Total and microbial nitrogen content of soft faeces decreased linearly with dietary NDF ($P=0.001$). Soft faeces of alfalfa A showed the highest value of total and microbial nitrogen (5.2 and 2.1%, respectively). Contribution of soft faeces to DM intake was not affected by type of diet, whereas contribution to N intake were quadratically influenced by diet, having alfalfas A and E the highest values (as average 22.9%). It can be concluded that the variation on chemical composition of alfalfa hays produce a change in variables that indicate fermentation characteristics such as caecal ammonia, VFA and microbial nitrogen concentration. However, this changes do not affect the caecal pH value.

Key Words: Alfalfa hay, rabbits, caecum, caecotrophy.

Introduction

Alfalfa hay is the most common source of fibre in rabbit diets. It is included in a high proportion in commercial diets (30% as average, in Spain). So, alfalfa meets a part of protein and energy but mainly fibre requirements in rabbits.

Chemical composition of alfalfa hay varies widely depending on several factors. These factors affect especially to the level and type of cell wall carbohydrates and lignin content. Several works have shown that level and type of fibre determine the characteristics of caecal fermentation (Carabaño et al, 1988; Motta, 1990; Fraga et al, 1991) and so the proliferation of pathogenic bacteria (Peeters, 1988).

The aim of this work has been to study the effect of chemical composition of alfalfa hay on chemical composition of caecal contents and soft feces production.

Materials and Methods

Diets

Five alfalfa hays out of ten were selected in order to obtain the greatest variability in fibre and protein composition, and were named A, B, C, D, E in increasing NDF order. Alfalfa hays were pelleted. Chemical analyses of these materials are shown in Table 1.

Caecal Measurement Trial

A group of 45 male and female New Zealand White x Californian rabbits (nine per diet), weighing from 1.50 to 1.60 kg, were allotted at random to the five diets. Animals were given ad libitum access to alfalfa hays as sole feeds throughout the experiment. After a period of 21 days animals were slaughtered

by cervical dislocation one hour before darkness, with an average weight of 2033, 1876, 2016, 2011 and 1878 g for alfalfas A, B, C, D and E respectively. Digestive measurements and caecal samples collection were done following the methodology described by Carabaño et al (1988). Caecal contents were analyzed in DM, NDF, N, ammonia and VFA concentration.

Caecotrophy Trial

A second group of 30 male and female New Zealand White x Californian rabbits weighing from 1.70 to 1.85 kg, were allotted at random (6 animals per diet) to the five diets. Animals were fed ad libitum only with the alfalfa hays throughout the experiment. Following a 10-d period of adaptation soft feces were collected according to Carabaño et al (1988). Soft faeces were analyzed in DM, N, CF, NDIN and microbial nitrogen concentration.

Analytical Methods

Chemical analysis were made using the method of Van Soest (1963) for ADF and ADL, Robertson and Van Soest (1981) for NDF, Goering and Van Soest (1970) for NDIN, and AOAC (1984) for DM, ash, N and CF. Uronic acids were determined by the m-phenyphenol method (Blumenkratz and Asboe-Hansen, 1973).

Caecal ammonia was determined using the autoevaluation distillation unit Kilab nitrolab-auto. Caecal VFA concentration was determined in a Hewlett-Packard (5710 A) gas chromatograph.

The microbial nitrogen was estimated using the total purine analysis method of Zinn and Owens (1980) as modified by Ushida et al (1985). The relative purine:N ratio of mixed (fluid and particle associated) bacteria isolated from caecal contents was assessed from rabbits fed with a commercial diet.

Statistical Analysis

Statistical analysis were performed using the GLM procedure of SAS (1985). Data from digestive measurements trial were corrected using as covariates average daily gain and slaughter weight, whereas data from caecotrophy trial were corrected using as covariates weight of the animal the day of soft faeces collection and the average daily intake three days before caecotrophy was avoided. Linear and quadratic treatment effects were tested to study the effect of chemical composition of diets.

Results and Discussion

Caecal Measurements Trial

Neutral detergent fibre content of alfalfa hays was selected as the main variable to study the effect of diet on all measurements, since it showed better correlation with them than CF, ADF, ADL, NSP or N.

Caecum, caecal contents and complete digestive tract weight increased linearly with dietary NDF proportion (Table 2). The differences between values observed for alfalfa hays A and E were 12, 35, and 25% for caecum, caecal contents and complete digestive tract weight, respectively. All in all, these effects in rabbits fed alfalfa E with respect to alfalfa A implied an impairment of five points of carcass performance.

Nitrogen and ammonia concentration of caecal contents decreased 14 and 30% respectively in alfalfa E compared to alfalfa A. The best correlated variable with caecal ammonia was dietary N content ($r=0.576$, $P<0.001$). The lower nitrogen content of alfalfa E with respect alfalfa A, together with a parallel increase of the proportion of NDIN might produce a lower availability of degradable nitrogen for microbial population. However, caecal ammonia concentration was high in all diets (> 15 mg N-NH₃ /100 ml). These high values might be accounted for a higher level of urea recycled in the caecum, as a consequence of the unbalance in the digestible energy/digestible protein ratio with respect to growth requirements of rabbits and (or) caecal microbial population.

Pattern of fermentation did not vary between diets (79.3, 6.6 and 14.1% as average for molar proportions of acetic, propionic and butyric acid, respectively). However, a quadratic tendency of dietary NDF content ($P=0.062$) was observed on total caecal VFA concentration. Alfalfas A and E showed the highest values, that could be related to its relatively greater soluble fiber and lower lignin content (alfalfa A) and its higher total fibre content in the caecum and longer caecal retention time of the digestive content (alfalfa E).

The pH values of caecal contents were not affected by type of diet. In spite of the high caecal ammonia concentration, pH (5.76 ± 0.06 , as average) could be enough to inhibit proliferation of pathogenic flora according to Peeters (1988). Caecal pH was related with total VFA ($r=-0.292$, $P=0.068$) and ammonia concentration ($r=0.281$, $P=0.079$). These results suggest the participation of others factors on the caecal acidity, such as the buffer capacity, the rhythm of VFA absorption or the caecal turnover rate.

Caecotrophy Trial

Type of diet influenced linear or quadratically all the variables measured except feed intake (which averaged 126.8 g DM/d), daily soft feces excretion (20.8 g DM), DM content (27.9%) and NDIN (0.47%) of soft faeces (Table 3).

Caecal turnover rate was estimated according to the following expression:

$$[\text{soft feces production}(\text{g DM/kg BW.d})/\text{caecal contents}(\text{g DM/kg BW})] \times 100$$

The values obtained were 117, 85, 72, 89 and 84% for alfalfas A, B, C, D and E, respectively, so that caecal contents of animals fed alfalfas with higher NDF content tended to remain longer in the caecum.

Contribution of soft faeces to N intake were affected quadratically by diet. Alfalfas A and E showed the highest values. According with the regression equation derived, a minimal value would be reached for a NDF dietary content of 456 g/kg (DM basis).

Dietary NDF proportion affected linear and quadratically the microbial nitrogen content of soft faeces. Alfalfa A showed the highest value. According with the regression equation derived, a minimal value would be reached for a NDF dietary content of 535 g/kg (DM basis). Soft feces from diet A contributed more to the essential aminoacid intake than those of diet B, C, D and E, particularly lysine, as Proto (1976) determined a high proportion (326 mg/g N) of lysine in soft faeces. This effect could be explained by the diminution of caecal turnover rate and by a decrease of fibre digestibility from diet A to diet E (Rocha et al 1994), although was not accompanied of a reduction of the VFA concentration. This fact could involve that caecal VFA concentration does not reflect accurately the microorganism activity.

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Table 1
Chemical composition of alfalfa hays (g/kg DM)

Item	Alfalfa hays				
	A	B	C	D	E
DM	902	919	918	923	937
CF	248	299	287	309	372
NDF	387	478	489	490	550
ADF	295	355	358	367	402
ADL	60	80	79	80	90
NSP	315	324	359	340	413
UA	74	73	73	75	79
N	35.2	35.5	33.4	28.3	27.0
NDIN	5.0	8.3	7.3	5.2	6.6

DM: Dry matter. CF: Crude fibre. NDF: Neutral detergent fibre. ADF: Acid detergent fibre. ADL: Acid detergent lignin. NSP: Non starch polysacharides. UA: Uronic acids. N: Nitrogen. NDIN: Neutral detergent insoluble nitrogen.

Table 2
Effect of type of alfalfa hays on caecal measurements

Item	Alfalfa hays					SE ¹	NDF ₁ ²
	A	B	C	D	E		
Caecum							
Organ wt, % BW	1.46	1.61	1.54	1.62	1.64	0.04	0.010
Content wt, % BW	5.17	6.52	6.47	5.85	7.16	0.25	0.001
Digestive tract, % BW	20.45	24.31	23.39	22.07	25.51	0.45	0.001
DM, %	19.6	19.4	19.4	20.3	18.8	0.27	NS ⁵
NDF, % DM	36.1	39.6	39.5	41.7	42.7	0.74	0.001
N, % DM	5.37	4.93	4.95	4.68	4.61	0.08	0.001
NH ₃ (mg N-NH ₃ /100 ml)	22.1	22.3	18.3	16.0	15.4	1.56	0.024
pH	5.69	5.83	5.82	5.70	5.76	0.04	NS
Total VFA ⁴ , mmol/l	88.5	80.2	84.4	82.9	87.3	3.14	NS ³
Acetic acid, %	78.8	78.8	80.1	79.4	79.6	0.45	NS
Propionic acid, %	6.63	7.10	6.44	6.36	6.46	0.20	NS
Butiric acid, %	14.6	14.01	14.0	14.2	13.9	0.38	NS

¹ SE: Mean standard error (n=9). ² NDF₁: Signification of linear effect of NDF
³ Quadratic tendency of NDF (P=0.062). ⁴ Volatile fatty acids. ⁵ NS: P>0.05

Table 3
Chemical composition and excretion of soft feces

Item	Alfalfa hays					SE ¹	NDF _i ²	NDF _c ³
	A	B	C	D	E			
soft feces excretion								
g DM/d	22.7	20.5	18.8	20.2	21.6	2.53	NS ⁷	NS
g DM/100 g BW d	1.19	1.07	0.90	1.06	1.13	0.13	NS	NS
Contribution to DM intake ⁴ , %	16.0	15.2	13.8	13.8	16.7	1.79	NS	NS
Contribution to N intake ⁵ , %	21.6	18.7	18.4	19.3	24.3	2.07	NS	0.025
DM, %	27.1	28.1	28.5	27.2	28.6	0.80	NS	NS
NDF, % DM	37.5	41.8	41.5	42.7	44.1	0.89	0.001	NS
N, % DM	5.20	4.56	4.81	4.29	4.52	0.11	0.001	NS
NDIN, % DM	0.49	0.40	0.49	0.49	0.50	0.03	NS	NS
N _{microbial} ⁶ , %	2.10	1.65	1.60	1.32	1.47	0.08	0.001	0.041

¹ Mean standard error (n=6)

² Signification of lineal effect of NDF

³ Signification of quadratic effect of NDF

⁴ As (Soft feces excretion (g DM/d)*100/(feed intake(g DM/d)+soft feces excretion (g DM/d)))

⁵ As (N excreted in soft feces (g/d)*100/(N ingested in feed (g/d)+N excreted in soft feces (g/d)))

⁶ Microbial nitrogen calculated from purine:N ratio (0.89 mg yeast RNA/mg total nitrogen of bacterial preparation)(n=6)

⁷ NS: P>0.05