

FEEDING RYEGRASS HAY TO GROWING RABBITS, A NOTE

FERNANDEZ-CARMONA J., CERVERA C., MOYA J., PASCUAL J.J.

Departamento de Ciencia Animal, Universidad Politécnica de Valencia, P.O. Box 22012, VALENCIA 46071, Spain.

ABSTRACT : Digestibility of ryegrass hay (18% crude protein and 18.9% crude fibre) was determined with 11 three-way crossbred New Zealand × Californian growing rabbits, using a 95% ryegrass diet +2% animal fat and 3% amino-acids, vitamins and minerals. Amino acids composition of diet and of the ryegrass was given. With the same diet, twenty-eight rabbits were used from 42 to 70 days of age to study the growth performance and carcass parameters. The ryegrass hay used in the present work showed high digestible energy

(10.3 MJ kg⁻¹ DM) and digestible protein (119 g kg⁻¹ DM) contents. Average daily gain, feed intake and live weight at 70 days were 36.7 g day⁻¹, 111 g DM day⁻¹ and 2042 g, respectively. Sex did not affect any of the carcass values. Weights of full gastrointestinal tract, liver and kidney fat were 504, 73 and 6.9 g. In conclusion, growth rate, feed conversion ratio (3.02 g g⁻¹ DM) and dressing percentage (53.3%) were satisfactory when this high forage diet was used.

RESUME : Note sur l'alimentation des lapins en croissance avec du foin de raygrass.

La digestibilité d'un foin de raygrass (18% de protéines et 18.9% de cellulose brute) a été estimée avec 11 lapins en croissance de type croisé 3 voies (Néo-Zélandais × Californien) en utilisant un aliment contenant 95% de foin de raygrass + 2% de graisses animales et 3% d'acides aminés, vitamines et minéraux. Les teneurs en acides aminés de l'aliment et du foin de raygrass sont fournies. Avec le même aliment, les performances de croissance entre 42 et 70 jours et les caractéristiques d'abattage ont été mesurées chez 28 lapins de même génotype. Le foin de raygrass utilisé avait une forte valeur

énergétique (10.3 MJ d'énergie digestible kg⁻¹ MS) et une teneur en protéines digestible également élevée (119 g kg⁻¹ de MS). La vitesse de croissance, l'ingestion d'aliment et le poids à 70 jours ont été respectivement de 36,7 g jour⁻¹, 111 g jour⁻¹ et 2042 g. Le sexe des lapins n'a exercé d'influence significative sur aucun des paramètres d'abattage. Les poids du tube digestif plein, du foie et du gras péri-rénal ont été de 504, 73 et 6,9 g respectivement. En conclusion, les auteurs considèrent que la vitesse de croissance, l'indice de consommation (3,02 en MS) et le rendement à l'abattage (53,3%), ont été pleinement satisfaisants pour un aliment expérimental contenant 95% de fourrage.

INTRODUCTION

A large scale use of forage in herbivorous animals feeding has been suggested as a suitable mean of saving raw materials for the human population, especially in those regions characterised by a low primary production. Therefore, rabbit production should be based on the use of high levels of forages, but in practice these ingredients are included by feed companies at only 30 to 50% in rabbit compound feeds. The remaining parts are mainly bran, cereals and protein concentrates (DE BLAS and MATEOS, 1998).

One forage widely used in ley farming is Italian ryegrass (*Lolium multiflorum*). This forage grass may be produced in a great variety of conditions but it has been scarcely used in rabbits feeding experiments. Some varieties are grown in Spain during winter so that the first cut can be done in early springtime.

According to the values available in some tables of feeding standards for ruminants published by institutions (the NATIONAL RESEARCH COUNCIL [NRC], 1989 and the INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE [INRA], 1978), Italian ryegrass has a chemical composition depending on its growth stage. Thus, the values reported in those publications are very variable: 84-130 g Ash, 9-36 g Ether Extract, 52-171 g Crude Protein, 197-360 g Crude Fibre, 3.5-7.0 g Ca and 2-3.4 g P (per kg DM).

No amino acid content is reported.

The only nutritive value of ryegrass in rabbits found in the literature was that reported by GRANDI (1983). The author determined the chemical composition of two varieties (*lenta* and *vejo*) of *Lolium perenne* and digestible energy content (7.6 and 8.5 MJ/kg DM respectively) using the indirect method with two levels of inclusion in the diet (7% and 14%).

It appears that the nutritive requirements of commercially crossed rabbits cannot be met exclusively with ryegrass, because of the physical limitation in ingestion. In addition, the protein level seems insufficient to cover the growing rabbit requirements, considering that the usual values for protein digestibility of forage rarely exceed 60%. The recommended levels of calcium and phosphorous for growing rabbits are 6 and 4 g/kg DM respectively (DE BLAS and MATEOS, 1998), which probably also exceed the ryegrass contents and, finally, ryegrass contains few sodium. However, with another quite pure forage diet, FERNANDEZ-CARMONA *et al.* (1998) reported a fairly good growth rate in rabbits from 35 to 70 days of age when fed a 96% lucerne diet.

Following the recommendations of the EGRAN group (PEREZ *et al.*, 1995), to increase the existing data on raw materials used in rabbit feeding, the aim of the present work was to determine the nutritive value and the performance of growing rabbits using a diet with 95% of ryegrass. It was considered that some of the obvious imbalances in energy, amino acids and minerals could be partially corrected. Economic

suitability of this type of forages based diet, which may be easily deduced from local prices of feedstuffs, was not considered.

MATERIAL AND METHODS

Animals

Thirty-four three-way crossbred (New Zealand × Californian) 42 days old rabbits were used in the study. They were produced by crossing the lines A, V and R obtained by the Genetics Unit of the Department of Animal Science of the Universidad Politécnica de Valencia (Spain). Rabbits were housed in individual cages.

Diet

Sun dried ryegrass (*Lolium multiflorum*) was used to determine the nutritive value of ryegrass hay in growing rabbits. After hay milling with a 3-mm sieve, diet was pelleted after addition of 20 g/kg of animal fat to obtain a stable non-hard pellet. The exact ingredient's list and chemical analysis of the experimental diet are summarised in tables 1 and 2.

Growth trial

The experiment took place between May and June in an air-cooled building, and the average weekly minimum dry bulb temperature ranged from 12 to 19°C.

Animals assigned at weaning to the experimental diet were previously fed on a commercial diet. Feed intake (*ad libitum*) and live weight were individually recorded during the experimental period (42 to 70 days). Daily feed intake was expressed per rabbit day⁻¹ or in terms of intake per mean metabolic weight of the animal, i.e. in g DM / kg^{-0.75} or kJ DE / kg^{-0.75}.

Digestibility trial

Apparent digestibility of dry matter (DM), organic matter, crude fibre (CF), crude protein (CP), and gross energy of the experimental diet were determined between 49 and 54 days of age, on 11 of the growing rabbits used in the growth trial. Rabbits were housed in metabolism cages and fed *ad libitum*. The adaptation period was 7 days and the faeces collection period was 5 days following the procedure of PEREZ *et al.* (1995), but faeces of all rabbits were mixed before analysis. This method leads to reliable average results, while saving a lot of work.

Table 1: Diet ingredients (g kg⁻¹ DM)

Ryegrass hay	950
Animal fat ¹	20
DL-Methionine	1.5
L-Lysine	1.5
Arginine	1.0
Dicalcium phosphate	21
Sodium chloride	2
Magnesium sulphate	0.15
Vitamins-minerals supplement ²	2.05
Robenidine ³	0.8

¹ 650 g lard, 250 g tallow and 100 g poultry fat per kg.

² Contains (g kg⁻¹): thiamin, 0.25; riboflavin, 1.5; calcium pantothenate, 5; pyridoxine, 0.1; nicotinic acid, 12.5; retinol, 2; cholecalciferol, 0.1; α -tocopherol, 39; phytylmenaquinone, 0.5; cyanocobalamin 0.006; choline chloride, 100; MgSO₄·H₂O, 7.5; ZnO, 30; FeSO₄·7H₂O, 20; CuSO₄·5H₂O, 3; KI, 0.5; CoCl₂·6H₂O, 0.2; Na₂SeO₃, 0.03.

³ Except in the last week

Table 2 : Composition of ryegrass and diet (g kg⁻¹ DM) and apparent digestibility coefficients (%) of the diet.

	Diet		Ryegrass
	Composition	Digestibility	Composition ¹
Dry matter (DM)	888	43.1	-
Ash	87.8	-	73 ²
Ether extract	47.8	64.4	30
Crude protein	175.3	67.1	180 ³
Crude fibre	179.8	21.4	189
Neutral detergent fibre	472.0	44.7	497
Acid detergent fibre	214.3	27.4	226
Acid detergent lignin	49.5	-	52
Gross energy (MJ kg ⁻¹ DM)	18.4	56.4	18.4 ⁴
Digestible energy (MJ kg ⁻¹ DM)	10.4	-	10.3 ⁴
Digestible protein (g kg ⁻¹ DM)	117.6	-	119

¹ values calculated from the diet

² premix contained 76 g ash kg⁻¹ DM

³ dl-methionine, l-lysine and arginine contain 58.5, 120.6 and 199.1% CP (as %N × 6.25), respectively

⁴ values calculated considering the following energy contents : animal fat (40 MJ GE and 25 MJ DE per kg DM) and amino acids (23 MJ GE and 21 MJ DE per kg DM).

Slaughter conditions

Rabbits were fed *ad libitum* until transport to the slaughterhouse in the morning, where the carcass measurements were obtained after removal of blood, skin, distal leg portions, urine bladder, digestive tract and organs located in the thorax and neck. In accordance with the criteria of BLASCO and OUHAYOUN (1996), commercial skin weight, full gastrointestinal tract weight and hot carcass weight were measured immediately after slaughtering, and then the carcasses were placed at 6°C. Recording weights of chilled carcass, kidney fat, liver and kidneys were performed after 24 h of refrigeration.

Analytical methods

Chemical analyses of diets and faeces were performed, according to the method of the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1996) for DM, ash, ether extract (EE), CP and CF and according to VAN SOEST *et al.* (1991) for neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) with a thermo stable amylase pre-treatment. Gross energy was measured using an adiabatic bomb calorimeter.

Amino acid composition of diet was determined following the method described by LIU *et al.* (1995) with some small modifications. Standard HCl hydrolysis was used for all amino acids except cystine and methionine, for which performic acid oxidation followed by HCl hydrolysis was used. Samples were derivatised by using the AccQ Tag Method (Waters Corp., Milford, MA). The derivatised samples were analysed in a HPLC system composed by two Waters 515 pumps (Waters Corp., Milford, MA), a Waters 474 scanning fluorescence detector (Waters Corp., Milford, MA), temperature control module (Waters) and manual injector. The separation was achieved in a Nova-Pack C18 column (3.9 x 150 mm) (Waters Corp., Milford, MA).

Statistical analysis

Because only one diet was used in this study no mean comparison was possible. Results are only presented with mean and standard deviation. Nevertheless, effect of sex on carcass parameters was studied with a one way variance analysis.

Table 4 : Rabbit growth and carcass parameters (N = 28)

	Mean	SD ¹
Growth parameters		
Live weight (g):		
at 42 days of age	1013	265
at 70 days of age	2042	206
Live weight gain (g day ⁻¹)	36.7	6.8
Feed intake (g DM day ⁻¹)	111.0	15.6
Feed intake (g DM kg ^{-0.75} day ⁻¹)	80.8	8.0
Energy intake (kJ DE kg ^{-0.75} day ⁻¹)	839	82.5
Feed conversion ratio (g DM g ⁻¹)	3.02	0.54
Carcass parameters		
Live weight at slaughter (LW, g)	2042	206
Skin weight (% LW)	11.8	1.4
Full gastrointestinal tract (% LW)	24.7	15.6
Hot carcass weight (g)	1125	146
Cold carcass weight (g)	1089	144
Dressing out percentage (%)	53.3	3.1
Liver weight (g)	73.4	14.5
Kidneys weight (g)	14.6	1.9
Perirenal fat weight (g)	6.9	3.5

Table 3 : Amino acid composition of the diet and ryegrass

	Diet	Ryegrass	
	g kg ⁻¹ DM	g kg ⁻¹ DM	g/ kg ⁻¹ CP
Lysine	7.32	5.82	32.4
Methionine	4.63	3.13	17.5
Cystine	2.67	2.67	14.9
Arginine	8.46	7.46	41.5
Threonine	6.31	6.31	35.1
Isoleucine	6.76	6.76	37.6
Valine	8.12	8.12	45.2
Aspartic acid	13.65	13.65	76.0
Serine	6.23	6.23	34.7
Glutamic acid	21.12	21.12	117.6
Glycine	7.19	7.19	40.1
Histidine	2.83	2.83	15.7
Alanine	9.61	9.61	53.5
Proline	11.44	11.44	63.7
Tyrosine	5.49	5.49	30.6
Leucine	11.15	11.15	62.1
Phenylalanine	7.54	7.54	42.0

RESULTS AND DISCUSSION

Nutritive value

The chemical composition and apparent digestibility coefficients of the diet are shown in Table 2, where the calculated values for ryegrass as an ingredient have been included.

The chemical composition values suggest, when compared to the values cited above from INRA (1978) and NRC (1989), that the hay used in the present work had a good quality, showing substantial DE and DP contents, even considering values for lucerne. Its composition is near to the values given by NRC (1989) for sundried ryegrass hay harvested at an early growth stage.

The DE value of ryegrass hay itself can be inferred if a fixed value for DE of animal fat and amino acids are assumed. The value for a similar fat from the same supplier can be calculated from the work of FERNANDEZ-CARMONA *et al.* (1998) as 23.8 MJ kg⁻¹ DM, similar to the mean value estimated by MAERTENS (1998) in his review (25 MJ kg⁻¹ DM). For synthetic amino acids, pure sources (95-99%) usually provide between 20.3 and 21.4 MJ DE kg⁻¹ DM (FEDNA, 1999). Therefore, considering 25 and 21 MJ kg⁻¹ DM for the animal fat and the synthetic amino acids respectively, the DE value of ryegrass hay can be deduced from the equation:

$$10.38 \text{ MJ/kg DM} = 0.950 \times \text{DE}_f + 0.020 \times 25 + 0.004 \times 21$$

from which the DE of ryegrass (DE_r) is 10.31 MJ kg⁻¹ DM, a value within the lowest limit of the normal range of commercial diets.

This DE content was higher than the values of 8.5 and 7.6 MJ kg⁻¹ DM given for the two varieties of perennial ryegrass (*Lolium perenne*) evaluated by GRANDI (1983). The differences could be due to several reasons: perennial and Italian ryegrass are different species of *Lolium*, and low inclusion levels of raw materials in a diet usually lead to great errors in the evaluation of their nutritive value. Moreover, the composition of ryegrass given by GRANDI (1983) suggests quite a lower quality (16-22 g EE kg⁻¹ DM, 105-122 g CP kg⁻¹ DM and 257-264 g CF kg⁻¹ DM) compared to that used in the present work.

Similarly, the values for CP and DP of ryegrass have been deduced to be 180 and 119 g kg⁻¹ DM respectively. The estimate relies upon a non-existing interaction between these ingredients and a digestibility coefficient of 100% for the synthetic amino acids. Moreover, in the 96% lucerne diet used by FERNANDEZ-CARMONA *et al.* (1998) fat addition did not seem to affect protein digestibility, but that possibility cannot be excluded in the present work. The apparent digestibility coefficient of the diet's protein was 67%, a normal value for forages and similar to the value reported by FERNANDEZ-CARMONA *et al.* (1998) for lucerne hay (64%). Higher values were reported by LEBAS (1987) for lucerne involved an industrially dehydrated product.

In fact, and taking into account the amino acid composition of its protein (Table 3), the ryegrass hay evaluated in the present work showed similar values, compared with the data available in the literature for lucerne hay (Mc DONALD *et al.*, 1995; FEDNA, 1999) and for other ryegrass hays (WINTERS *et al.*, 2000). Ryegrass shows slightly higher values for the sulphur amino acid content (32.3 vs. 24.5-25.6 g kg⁻¹) although lower values for the lysine content (32.4 vs. 42.9-62.8). In any case, the content of sulphur amino acids may cover the requirements for growing rabbits but not that of lysine (De BLAS and MATEOS., 1998).

Growth trial

From the 34 initial rabbits, 4 died during the trial (11.8%) and results from 2 additional rabbits were discarded for registration problems. The 28 remaining rabbits (13 males and 15 females) were used to study the influence of the ryegrass diet on the growth performance and carcass parameters as shown in Table 4.

The most comparable results corresponding to an «all forage diet» were obtained by (FERNANDEZ-CARMONA *et al.* (1988) with similar life weight gain (37.3 g d⁻¹) and slaughter weight at 70 days of age (2160 g), obtained with a diet composed of 96% lucerne + 1% animal fat and 3% of premix

It may be suggested that the live weight gain of rabbits fed on a commercial diet in the same management conditions would have been higher, in the same way that in the work published by FERNANDEZ-

CARMONA *et al.* (1998). In this work, the best performances were recorded with the control diet: 40.3 g day⁻¹ and 2290 g at 70 days. Under the same conditions of farm, animals and ambient temperature, CERVERA *et al.* (1997) reported an average gain of 38.2 g day⁻¹ when rabbits from 35 to 84 days of age were fed on three high-energy balanced diets.

With regard to those works where ryegrass has been used as main forage, ryegrass hay has been included only three times, as far as we know, in diets for growing rabbits, at levels not higher than 50%. Substitution of ryegrass by other forages induced comparable results (GRANDI, 1983; PAYNE *et al.*, 1983; RANDHIR *et al.*, 1994).

Feed conversion ratio value was about 0.6 units better than that reported by FERNANDEZ-CARMONA *et al.* (1998) for the lucerne diet. This value can be compared to others obtained with commercial diets: 3.06 is the value given by MAERTENS and VILLAMIDE (1998) on a fresh matter basis in similar conditions of initial and final weight, with a weight gain of 44.7 g day⁻¹. Mean mortality rate was about 11%, but from the 42nd day of age.

Carcass.

Sex did not affect any of the carcass values. The gastrointestinal tract including the reproductive organs has sometimes been found heavier in females (LOPEZ *et al.*, 1988), but in the present work the difference between sexes was not significant.

Adaptation to high fibrous diets and relatively low DE concentration increased size of the digestive system (Table 4). Effectively the proportion of full gastrointestinal tract is 24.7% of LW in the present study but only 18% for balanced concentrate diets (OUHAYOUN, 1998). Full gastrointestinal tract weight and its related value, dressing yield were very similar to those reported by FERNANDEZ-CARMONA *et al.* (1998) for the 96%-alfalfa diet (21.3% and 55.4% respectively). Dressing out percentage (DoP) was clearly lower than the 58-60% values frequently found in other experiments with rabbits weighing 2 kg. It should be related to the heavier gut, including digestive contents, and to thoracic organs removal. DoP does not vary when the live weight gain was similar for diets very different in fibre (OUHAYOUN, 1998).

Liver represented 7% of the carcass weight, closely coincident with the figure obtained by LEBAS *et al.* (1982) and similar to other authors. Earlier reports on variation of liver weight with diet were not conclusive (OUHAYOUN *et al.*, 1986).

Finally, fat deposit is a variable very sensitive to variation of energy intake. When comparing the present work and that of FERNANDEZ-CARMONA *et al.* (1998), we could suggest that in the present study a lower energy intake (839 vs 902 kJ DE kg^{-0.75}) allowed the same growth rate (37 g day⁻¹), but a lower kidney

fat deposition (6.9 vs 11.4 g with the 96% lucerne diet).

In conclusion, a diet with 95% of ryegrass hay could support a growth rate, feed efficiency and dressing percentage of approximately 90% of the corresponding values found in good conditions with commercial diets. Nevertheless it must be pointed out that this conclusion is related to the particular characteristics of the ryegrass hay used. The response to hays of different quality might have been significantly different.

Acknowledgment : The present work was supported by a grant from CICYT (AGF97-1139).

Received : March 13th, 2001

Accepted : September 18th, 2001

REFERENCES.

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1984. Official Methods of Analysis (14th ed.). *Association of Official Analytical Chemists*, Washington, DC.
- BLASCO A., OUHAYOUN J., 1996. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Science*, **5**, 71-75.
- CERVERA C., BLAS E., FERNANDEZ-CARMONA J., 1997. Growth of rabbits under different environmental temperatures using high fat diets. *World Rabbit Science*, **6**, 237-240.
- DE BLAS C., MATEOS G.G., 1998. Feed formulation. in: *The Nutrition of the Rabbit*, De Blas C. and Wiseman J. (Eds), CABI Publishing, Wallingford, UK, 241-253
- FEDNA, 1999. Normas FEDNA para la formulación de piensos compuestos. De Blas, C., Mateos, G.G., Rebollar, P.G. Ed: *Fundación Española para el Desarrollo de la Nutrición Animal*, Madrid, 496.
- FERNANDEZ-CARMONA J., BERNAT F., CERVERA C., PASCUAL J.J., 1998. High lucerne diets for growing rabbits. *World Rabbit Science*, **6** (2), 227-240.
- GRANDI A. 1983. Lolium perenne L. (var. lenta e var. vejo) come alimento alternativo per il coniglio. *Rivista di Coniglicoltura*, **20** (12), 49-51.
- INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE (INRA), 1978. Alimentation des Ruminants. INRA Publications (Publisher).
- LEBAS F., 1987. La luzerne déshydratée et le lapin. *Cuni- Sciences*, **4**, 11-22.
- LEBAS F., LAPLACE J.P., DROUMENQ P., 1982. Effets de la teneur en énergie de l'aliment chez le lapin. Variations en fonction de l'âge des animaux et de la séquence des régimes alimentaires. *Annales de Zootechnie*, **31**, 233-256.
- LIU H.J., CHANG B.Y., YAN H.W., YU F.H., LIU X.X., 1995. Determination of amino acids in food and feed by derivation with 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate and reversed-phase liquid chromatographic separation. *Journal of AOAC International*, **78**, 736-744.
- LÓPEZ A., DELTORO J., CAMACHO J. (1988) Quantitative growth of rabbit organs. in: *Proceedings of the 4th World Rabbit Congress, Budapest October 1988, vol II*, 352-360.
- MAERTENS L. 1998. Fats in rabbit nutrition: a review. *World Rabbit Science*, **6**, 341-348
- MAERTENS L., VILLAMIDE M.J. 1998. Feeding standards for intensive production in: *The Nutrition of the Rabbit*, De Blas C. and Wiseman J. (Eds), CABI Publishing, Wallingford, UK, 263-271
- MC DONALD P., EDWARDS R.A., GREENHALGH J.F.D., MORGAN C.A., 1995. Animal Nutrition. Ed: A.W. Longman. *Edinburgh Gate, Harlow*, 607.
- NATIONAL RESEARCH COUNCIL (NRC), 1988. *Nutrient requirements of dairy cattle, 8th. ed. National Academy Press, Washington DC.*
- OUHAYOUN J., LEBAS F., DELMAS D., 1986. La croissance et la composition corporelle du lapin: Influence des facteurs alimentaires. *Cuni-Sciences*, **3**: 7-21.
- OUHAYOUN J., 1998. Influence of the diet on rabbit meat quality. In: *The Nutrition of the Rabbit*, De Blas C. and Wiseman J. (Eds), CABI Publishing, Wallingford, UK, 177-195
- PAYNE M., BRYANT M.J., OWEN E., CAPPER B.S., WOOD J.F. MACHIN D.H., BUTCHER C., 1983. The effect of diets containing 50% roughage on performance and digestibility in growing rabbits. *Tropical Animal Production*, **8**, 269-275.
- PEREZ J.M., LEBAS F., GIDENNE T., MAERTENS L., XICCATO G., PARIGI-BINI R., DALLA-ZOTTE A., COSSU M.E., CARAZZOLO A., VILLAMIDE M.J., CARABAÑO R., FRAGA M.J., RAMOS M.A., CERVERA C., BLAS E., FERNANDEZ-CARMONA J., FALCAO E CUNHA M.L., BENGALA FREIRE J., 1995. European reference method for in vivo determination of diet digestibility in rabbits. *World Rabbit Science*, **3**, 41-43.
- RANDHIR S., SAWAL R.K., BHASIN V., BHATIA D.R., SINGH R., 1994. Nutrient utilisation by rabbits on diets containing kudzu hay. *Indian Journal of Animal Nutrition*, **11**, 255-258.
- VAN SOEST P.J., ROBERTSON J.B., LEWIS B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, **74**, 3583-3597.
- WINTERS A.L., COCKBURN J.E., DHANOA M.S., MERRY R.J., 2000. Effects of lactic acid bacteria in inoculants on changes in amino acid composition during ensilage of sterile and non-sterile ryegrass. *Journal of Applied Microbiology*, **89**, 442-451.