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EFFECTS OF TYPE AND LEVEL OF FIBRE ON DIGESTIVE PHYSIOLOGY AND PERFORMANCE IN REPRODUCING AND GROWING RABBITS

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ABSTRACT: The study aimed to evaluate the effect of level and type of fibre on performance and digestive traits of lactating does and growing rabbits. Two lactation diets were formulated to contain a similar level of NDF (29%): a mixture of alfalfa hay and wheat straw in diet L1 was substituted with apple pulp in diet L2. Three fattening diets were formulated: diet F1 was the same than diet L1 whereas F2 and F3 had higher level of NDF (33 and 36%, respectively) with a mixture of wheat straw and alfalfa hay in diet F2 substituted by apple pulp in diet F3. A total of 110 does and their litters were controlled during two consecutive lactations from 21 to 35 d. At weaning (35 days of age) 480 rabbits were housed collectively (four per cage) and 180 individually. Another 54 rabbits were slaughtered to determine digestive traits at 45 days of age. At 35 days of age, L1 rabbits showed 5.9 and 6.9% higher daily feed intake and body weight than L2 rabbits. In the fattening period (35 to 63 days), L1 rabbits showed a higher growth rate but a lower feed efficiency than L2 animals. In the fattening period, F1 rabbits showed a 5.9% higher weight gain and 8.8% lower feed conversion than F2 animals, whereas animals fed diet F2 had a higher weight gain (7.6%) and a worse feed conversion (6.5%) than animals fed diet F3. The highest apparent ileal digestibility of DM values was obtained for diets F1 and F3. Villi length tended to be longer (P<0.10) in animals fed the diet F1 and F3. No significant influence of treatments was detected on caecal pH, anaerobic bacteria counting or rabbit mortality (only 0.9% on average). In conclusion the substitution of a mixture of wheat straw and alfalfa hay by apple pulp in isofibrous diets increases feed efficiency but also impairs feed intake and growth rate. Furthermore, in a context of low mortality, diets with 29-30% NDF would lead to better performances in the fattening period than diets containing 33-36% NDF levels.

Key words: Type of fibre, level of fibre, digestion, performance, rabbits.

INTRODUCTION

Insoluble fibre is the dietary fraction most related to digestive troubles in rabbits, as it is the most important factor in regulating rate of passage and microbial growth (De Blas *et al*., 1999; García *et al*., 2000; Gidenne, 2003). A minimum level of 34% of NDF is recommended to minimize the accumulation of *digesta* in the caecum and maximize performance during the whole fattening period (De Blas and Mateos, 1998). However, the recommendations for the post-weaning period might be different since the colonization of gut microbiota (Gouet and Fonty, 1979; Padilha *et al*., 1995) and the development of digestive capacity and the gut associated lymphoid tissue are not completed yet (Lebas *et al*., 1971; Lanning *et al*., 2000; Dasso *et al*., 2000).

Otherwise, the inclusion of soluble and fermentable fibre in the diet decreased the caecal pH and increased the total concentration of volatile fatty acids (Fraga *et al*., 1991, García *et al*., 1993; Carabaño *et al*., 1997), which might help to control pathogenic flora and to prevent digestive disorders (De

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Blas *et al.*, 2002). However, it also increased total mean retention time and reduced feed intake (Pairet *et al.*, 1986; Fraga *et al.* 1991, García *et al.*, 1993, Carabaño *et al.*, 1997; Falcao-e-Cunha *et al.*, 2004). The opposite effect has been observed with the dietary inclusion of insoluble and low fermentable fibre (Fraga *et al.*, 1991; Motta-Ferreira *et al.*, 1996; García *et al.*, 2000) or by increasing ADL content (Gidenne and Perez, 1994; Nicodemus *et al.*, 1999; García *et al.* 2002a). Accordingly, a balance between soluble and insoluble fibre seems to be advisable to maximize fattening performance. A maximum digestible fibre (pectins + hemicellulose)/ADF ratio of 1.3 has been recommended to avoid the increase of sanitary risk (Gidenne, 2003), defined as the sum of the mortality and the proportion of animals with a low growth rate, usually observed in morbid animals. However, the use of diets with higher values of this ratio in the post-weaning period reduced the mortality in other studies (Gómez-Conde *et al.*, 2004; Soler *et al.*, 2004; Fabre *et al.*, 2006). This effect has been related to the beneficial influence of soluble fibre on the mucosa integrity (Gómez-Conde *et al.*, 2004) and to the reduction of the frequency of detection of enteric pathogens as *Clostridium perfringens* (Gómez-Conde *et al.*, 2006). A correct evaluation of soluble fibre requirements should be then made taking into account its influence on the intestinal health and on the productive performance. Furthermore, the effects might differ if supply starts in the lactation period, as the microbial colonization of the intestinal tract and the development of the fermentative area become relevant when the animals begin to eat solid feed (18-20 days of age).

The aim of this work was to evaluate the effects of level of inclusion of soluble and insoluble fibre, by measuring animal performance and digestive traits in rabbit does and in growing rabbits from 21 to 35 and from 35 to 63 days of age.

MATERIAL AND METHODS

Diets

Two isofibrous and isonitrogenous lactation diets were made by substituting a mixture of alfalfa hay and wheat straw (diet L1) with apple pulp (diet L2), which increased both soluble fibre and lignin dietary content (Tables 1 and 2). Diet L1 was also used as a fattening diet (F1). Another two fattening diets were formulated with a higher level of fibre, by increasing the level of wheat straw, sunflower meal and wheat bran (diet F2) or by substituting alfalfa hay and wheat straw of diet F2 with apple pulp (diet F3). Diets F2 and F3 had a similar level of total fibre content, but different proportions of insoluble (NDF) and soluble fibre (Tables 1 and 2). In order to determine apparent ileal digestibility, 5 g/kg DM of alfalfa hay marked with Yb_2O_3 were included in all diets according to the procedures described by García *et al.* (1999). All the experimental diets were formulated according to the nutrient recommendations of De Blas and Mateos (1998). Neither feeds nor drinking water were medicated at any moment of the trial.

Animals and housing

Multiparous New Zealand × Californian rabbit does and their litters were assigned at random to the experimental treatments. Rabbit does and litter performance was recorded in two consecutive lactating periods from 21 to 35 days of age (lactation trial) and growing rabbit performance was recorded from 35 to 63 days of age (fattening trial).

All trials were performed in the Fattening Rabbit Unit at the Poultry and Rabbit Research Centre facilities of NUTRECO. Part of the animals was housed individually in flat-deck cages measuring 35 cm × 46 cm × 30 cm high. Polyvalent cages (38×100×30 cm) were used to house groups of four rabbits in the collective trial. Heating and forced ventilation systems allowed the building temperature to be maintained at 19 ± 4ºC in the two trials. Rabbits were handled according to the principles for the care of animals in experimentation published by the Spanish Royal Decree 1201/2005.

Table 1: Ingredient and chemical composition of experimental diets

	Diet			
	L1/F1	L2	F2	F3
Ingredients, %				
Alfalfa hay	23.5	17.5	12.0	6.1
Wheat straw	6.0	2.0	17.5	9.5
Apple pulp	-	9.88	-	13.77
Barley grain	26.0	26.0	19.0	19.0
Wheat bran	24.0	24.0	24.0	24.0
Sunflower meal, 30% CP	5.0	5.0	12.0	12.0
Soybean meal, 44% CP	11.0	11.0	11.0	11.0
Soybean oil	2.88	2.88	2.88	2.88
Sodium chloride	0.5	0.5	0.5	0.5
Monocalcium phosphate	0.5	0.5	0.5	0.5
HCL-lysine	0.02	0.07	0.09	0.15
L-Threonine	0.05	0.09	0.08	0.12
DL-Methionine	0.05	0.08	0.05	0.08
Premix[1]	0.5	0.5	0.5	0.5
Chemical composition, % as fed				
DM	89.2	89.2	89.1	88.7
Ash	5.82	5.63	5.37	5.20
CP	16.8	16.7	16.7	16.8
Starch	21.5	21.9	19.5	19.1
NDF	29.1	28.3	36.1	33.3
ADF	16.0	15.9	18.6	19.4
ADL	3.57	4.04	4.25	5.14
Soluble fibre[2]	9.6	11.2	8.51	11.0
Digestible energy, MJ/kg[3]	10.8	11.3	9.92	10.6
Lysine[3]	0.781	0.786	0.776	0.776
Sulphur aminoacids[3]	0.591	0.592	0.599	0.608
Threonine[3]	0.637	0.665	0.653	0.658

[1] Included 0.40% vitamin-mineral premix and 0.10% Robenidine (6.6%). [2] Estimated as (100 - moisture - ash - CP - ether extract - NDF - starch - sugars); sugars where estimated according to FEDNA (2003). [3] Values estimated according to FEDNA (2003).

Lactation trial

One hundred and ten (55 per diet) lactating does were used. Nest access was restricted to 30 minutes per day up to 21 days of age. Does were artificially inseminated 45 minutes after suckling at the 11th day of lactation and kits were weaned at 35 days of age. Ovulation was induced with 0.2 ml of GnRH. Non pregnant does were not inseminated up to the following reproductive cycle. Litters were not equalized. Does received either of the experimental diets during the period from 21 to 35 days of lactation. The rest of the time does were fed with a non medicated standard lactation diet, similar to diet L1. Does were given *ad libitum* access to feed. Young rabbits had free access to maternal diet.

Table 2: Chemical composition of the main fibre sources (% as fed)

	Alfalfa hay	Wheat straw	Apple pulp
Dry matter	93.6	92.6	89.9
Crude protein	15.4	3.0	5.9
NDF	40.2	71.5	46.1
ADF	31.3	43.4	33.1
ADL	8.8	6.3	11.1
Soluble fibre[1]	10.5	2.5	24.5

[1]Estimated as (100 - moisture - ash - CP - ether extract - NDF - starch - sugars); sugars where estimated according to FEDNA (2003)

Performance traits (litter size and litter weight at 21 days of age and at weaning, young and rabbit doe mortality) were recorded per cage throughout the two experimental periods. Feed consumption from 21 to 35 days of lactation and weight of does between parturitions and on day 21st of lactation were also recorded.

Fattening trial

Six hundred and sixty weaned rabbits (220 per fattening diet) were blocked by litter. They were assigned to the treatments so that half of the animals fed each fattening diet received previously each of the two lactation feeds. One hundred and eighty of the weaned rabbits (60 per fattening diet) were individually housed. Another 480 (160 per fattening diet) were caged collectively in groups of four. Growth rate, feed intake and mortality were recorded from 35 to 63 days of age.

Digestibility trial

Fifty four weaned rabbits (18 per diet) were blocked by litter and assigned at random to the three fattening diets to determine ileal digestibility of DM and CP, several digestive traits and total anaerobic bacteria in the caecum. Following a 10-d adaptation period, animals were slaughtered by cervical dislocation between 19.00 and 21.00 h. The last 20 cm of the ileum were taken and the ileal contents were removed, frozen and freeze-dried. The samples were then ground and, because of the small quantity available, they were pooled in groups of two rabbits of the same treatment to analyse CP and ytterbium concentration. Ytterbium concentration of experimental diets (Ybd) and ileal digesta (Ybid) were analysed to calculate DM and CP ileal apparent digestibility (DM_{id} and CP_{id} according to the following equation):

$$DM_{id} = [1 - Ybd / Ybid] \times 100.$$
$$CP_{id} = [1 - Ybd \times \text{ileal CP concentration}/Ybd \times \text{dietary CP concentration})] \times 100.$$

In all the animals slaughtered the gastrointestinal tract was removed and weighed. Stomach and caecum were weighed separately with and without their contents. The pH of caecal and stomach contents was determined. A sample was taken from the middle part of the jejunum of each animal to determine mucosa histology. The samples were placed in a 10%-buffered neutral formaldehyde solution (pH 7.2 to 7.4) and were gradually dehydrated with increasing concentrations of ethyl alcohol (50 to 100%). These dehydrated specimens were first embedded in paraffin, prepared by sectioning at 6 μm, and stained with hematoxylin and eosin. The sections were analysed under a light microscope (Olympus BX40, Olympus Optical Co., 20097, Hamburg, Germany) to determine their morphometric index by computer-assisted image analysis (The ImageJ v 1.26. Wayne Rasband, National Institutes of Health, Bethesda, MD 20892, USA). Villi height (from the top of the villi to the villi crypt junction) at three cross sections was measured according to Hampson (1986) from the mean value of 30 vertically-oriented villi per animal.

Caecum content samples were aseptically taken and transferred into sterile tubes. The sterile tubes were then placed into GENbag anaer 45534 (bioMérieux S.A., Marcy I'Etoile, France) for further determination by keeping the viability of anaerobic microflora. Then, 1 g from each sample was aseptically removed from the sterile tube and transferred to another sterile tube being diluted from 10^{-1} to 10^{-9} in Buffered Peptona Water BK018HA (Biokar Diagnostic, Zac Dether-Allonne-Beauvais, France) and mixing gently with vortex. From each of the 10^{-8} and 10^{-9} ileum dilution, 0.1 ml was plated onto a Anaerobe Basal Agar CM0972 (Oxoid Ltd., Basingstoke, Hamphsire, UK) supplemented with 5% sterile Defibrinated Horse Blood SR0050 (Oxoid Ltd., Basingstoke, Hamphsire, UK).

Analytical Methods

Chemical analysis of diets was made using the procedure of Van Soest *et al*. (1991) for NDF, ADF and ADL. Methods of the AOAC (2000) were used for DM, ash, CP and starch determinations. Sugars where estimated according to FEDNA (2003) and soluble fibre calculated as reported in Table 1. Ytterbium concentration of diets and ileal digesta were analyzed by atomic absorption spectrometry (Smith Hieftje 22, Thermo Jarrel Ash, MA, USA) using predosed samples to prepare common matrix standards. Previously, samples were ashed (600°C) and then digested by boiling with a solution of 1.5 mol/l HNO_3 and KCl (3.81 g/l).

Statistical Analysis

Data of lactation trial were analysed as a completely randomised design with type of lactation diet as the main source of variation by using the General Linear Model (GLM) procedure of SAS (1990). Since the period of lactation did not influence any of the traits studied, data from both periods were averaged for statistical analyses. The effects of lactation diet, fattening diet and type of cage and their interaction were included in the model for fattening performance traits. The cage was considered as the experimental unit in the collective trial. Mean comparisons among fattening diets were made using a *t*-test.

RESULTS

The effect of lactation diet on the performance of adult and young rabbits before weaning is shown in Table 3. The type of feed did not affect either body weight or mortality of rabbit does, litter size at 21 or 35 days of lactation, or the mortality of young rabbits. However, feed intake per cage ($P < 0.02$)

Table 3: Effect of lactation diet on performance of rabbit does and suckling rabbits

	Diet			
	L1	L2	SEM[1]	*P*-value
Rabbit does weight, g				
at 21 d of lactation	4851	4880	54	
at 35 d of lactation	4673	4716	56	
Rabbit does mortality, %	0.0	2.1	1.4	
Litter size at 21 d	8.1	8.3	0.2	
Litter size at 35 d (weaning)	8.1	8.2	0.2	
Kit mortality (21 to 35 d), %	0.3	0.9	0.3	
Average kit weight, g				
at 21 d of age	361	352	5.68	
at 35 d of age	863	807	10.9	<0.001
Feed intake (21 to 35 d), g/cage/d	572	540	10	0.02

[1]SEM = standard error of means (n = 55)

Table 4: Effect of fattening diet on ileal digestibility, jejunal morphology and number of caecal anaerobic bacteria in young rabbits of 45 days of age

	Diet			SEM[1]	Contrast *P*-value*	
	F1	F2	F3		F1 *vs*. F2	F2 *vs*. F3
AIDDM[2]	60.9^{a}	55.2^{b}	60.1^{a}	1.4	0.03	0.05
AIDCP[3]	79.1	76.9	78.8	0.8		
Villi length, m	714	619	704	37	0.06	0.07
Crypt depth, m	89.0	92.1	97.7	4.0		
Villi length/crypt depth ratio	8.42^{a}	7.01^{b}	7.46^{ab}	0.41	0.05	
No. of anaerobic bacteria[4]	4.06	5.41	3.97	0.78		

*Contrast: F1 *vs*. F2 (level of fibre), F2 *vs*. F3 (source of fibre). [a,b]Means within the same row with different superscripts differ (P<0.05). [1]SEM = standard error of means (n=18, except for ileal digestibility, where n=9). [2]AIDDM = apparent ileal dry matter digestibility (%). [3]AIDCP = apparent ileal crude protein digestibility (%). [4]Colonies forming units x 10^9 /g of caecal content.

and weight of young rabbits at weaning (P < 0.01) decreased by 5.6 and 6.5% respectively, when apple pulp was included in the diet L2 with respect to diet L1.

The effect of treatments on digestion traits is presented in Tables 4 and 5. An increase of dietary level of NDF from 29% (diet F1) to 36% as-fed when traditional sources of fibre were used (wheat straw, sunflower meal and wheat bran, diet F2), decreased apparent ileal digestibility (AID) of DM (by 9.4%, P = 0.03), jejunal villi length (by 13.3%, P = 0.06), and villi length to crypt depth ratio (by 16.7%, P = 0.05). Substitution of a mixture of wheat straw and alfalfa hay in diet F2 with apple pulp (diet F3) at high level of NDF (33% as-fed) increased AID of DM (by 8.9%, P = 0.05) and tended (P = 0.07) to increase villi length up to a value similar to that obtained with the low-fibre diet (F1). Treatments did not influence either AID of CP or number of caecal anaerobic microorganisms, although microbial density was negatively correlated with AID of DM. Rabbits that were fed diet L1 before weaning had a lower weight of the caecum (by 6.7%, P = 0.01), and of the whole digestive tract (by 6.4%, P = 0.002) at 45 days of age, than those receiving diet L2. However, type of fattening feed did not affect any of these traits, neither caecal pH.

The rabbit performance during fattening trial (35 to 63 d) is presented in Table 6. Animals caged individually had a higher growth rate (by 6.3%, P < 0.001), feed intake (by 11.4%, P < 0.001) and a worse feed conversion rate (by 4.9%, P < 0.001) than those caged in groups. Feeding diet L2 instead of diet L1 before weaning also led to lower weights, both at 35 (by 6.5%, P < 0.001) and at 63 days of age (by 3.2%, P< 0.001). An increment of level of fibre in the fattening diet implied a decrease of daily growth rate, especially when apple pulp was included in the diet replacing a mixture of wheat straw

Table 5: Effect of diets on weight of digestive contents and caecal pH at 45 d of age

	Lactation diet		Fattening diet			SEM[1]	Contrast *P*-value*		
	L1	L2	F1	F2	F3		L1 *vs*. L2	F1 *vs*. F2	F2 *vs*. F3
Gut weight, %BW	17.6	18.8	18.1	18.1	18.3	0.5	0.002		
Caecum weight, %BW	6.95	7.45	7.37	7.05	7.19	0.23	0.01		
Caecal pH	5.71	5.66	5.66	5.71	5.69	0.05			

*Contrasts: L1 *vs*. L2 = Effect of lactation diet fed before weaning, F1 *vs*. F2 = Effect of level of fibre in fattening diets, F2 *vs*. F3 = Effect of source of fibre in fattening diets. Interactions of contrasts 1 x 2 and 1 x 3 were non significant (P>0.15). [1]SEM = standard error of means (n=18)

Table 6: Effect of type of diet on fattening performance from 35 to 63 days of age

	Housing system (HS)[1]		Lactation diet (LD)		Fattening diet (FD)				*P*-value[3]		
	Individual	Collective	L1	L2	F1	F2	F3	SEM[2]	HS	LD	FD
Initial weight at 35 d, g	829	843	864	808	837	837	834	13		<0.001	
Final weight at 63d, g	2163	2096	2165	2095	2213[a]	2136[b]	2041[c]	28	0.001	<0.001	<0.001
Daily growth rate, g	47.6	44.8	46.4	45.9	49.1[a]	46.4[b]	43.1[c]	0.6	<0.001		<0.001
Daily feed intake, g	121	108	116	113	117[b]	121[a]	106[c]	2	<0.001	0.03	<0.001
Feed conversion	2.54	2.42	2.50	2.45	2.38[c]	2.61[a]	2.45[b]	0.02	<0.001	0.02	<0.001
Mortality, %	0.5	1.3	1.0	0.8	1.1	1.3	0.3	1.0			

[a,b,c]Means for fattening diets within the same row with different superscripts differ (P>0.05). [1]Individual housed (n=60 cages per fattening diet); Collective housed (n=40 cages per fattening diet). [2]SEM = standard error of means. [3]Interactions HSxFD and LDxFD were non significant (P>0.15)

and alfalfa hay. Feed intake and feed conversion rate were higher (by 3.4 and 9.6%, respectively) in animals receiving diet F2 with respect to diet F1. Inclusion of apple pulp decreased intake (by 12.4%, $P < 0.05$), but also feed conversion rate (by 6.2%, $P < 0.05$)) in animals fed the diets with the highest level of fibre (F2 and F3). Mortality rate was very low in this experiment (less than 1% as average), and was not affected by any of the treatments studied. Neither the interaction cage density x type of fattening diet or that of lactation × fattening diet affected significantly ($P > 0.15$) any of the traits studied.

DISCUSSION

Substitution of traditional sources of fibre with apple pulp decreased feed consumption both during the lactation and the fattening period. A lower feed intake during lactation also had a slight carry over effect on fattening intake, which might be a consequence of a lower weaning weight and/or a higher accumulation of digesta in the caecum at 42 days of age. Previous works have also shown that dietary supplementation with soluble fibre from citrus or beet pulps decreased intake (Fraga *et al.*, 1991; García *et al.*, 1993; Carabaño *et al.*, 1997; Falcao-e-Cunha *et al.*, 2004). The effect was more important when soluble fibre replaced starch and was then associated to an increase of total dietary fibre content. Reduction of intake in our study was moderate, as diets were isofibrous and inclusion of soluble fibre with apple pulp was parallel to a higher lignin concentration, which has been proven to increase feed intake in lactating and fattening rabbits (Perez *et al.*, 1994; Nicodemus *et al.*, 1999; García *et al.*, 2002a). Combined incorporation of soluble fibre and lignin also implied a lack of effect of type of fattening feed on caecal acidity and on weight of caecum at 42 days of age, which might have additionally reduced intake according to García *et al.* (2002b). The effects of inclusion of apple pulp on reproductive and fattening performance were smaller or not significant, as a higher digestion efficiency compensated partial or totally the impairment of ingestion and allowed for an improvement of feed conversion rate during the fattening period (-6.2%). Several studies on rabbits have determined a relatively high energy value of soluble fibre sources (as beet and citrus pulps) compared to other fibrous concentrates (Martínez and Fernández, 1980; Maertens and De Groote, 1984; De Blas and Villamide, 1990). Later works reviewed by De Blas *et al.* (1999) suggest that soluble fibre accounts for most of the total cell wall content digested in rabbits, where the short caecal fermentation time limits the fibrolitic activity of the gut flora in this species (Marounek *et al.*, 1995; Falcao-e-Cunha *et al.*, 2004). This effect was independent of the dietary ADL content, because of the low degree of chemical binding between both cell wall fractions.

A decrease of insoluble fibre concentration (NDF) from 36 to 29% in diets based on traditional sources of fibre also led to a slight decrease of feed consumption and to a greater increase of ileal DM digestibility. As a consequence, rabbits fed the diet with the lowest level of fibre had the highest growth rate and the lowest feed conversion among the fattening diets studied, which agrees with previous results (Gutiérrez *et al.*, 2002) obtained with rabbits weaned at an earlier age (25 days).

Mortality during fattening was very low in our study, both in individually and collectively caged animals, so that the hypothetical effects of supplementation with soluble or insoluble fibre could not be tested. Anyway, the results indicate that inclusion of apple pulp in highly fibrous fattening feeds tended to improve jejunal mucosa integrity and to reduce the flow of DM towards the fermentative area, which was reflected in a 27% (but non significant) decrease of number of caecal bacteria. Similar effects were observed when decreasing level of insoluble fibre in diets based on traditional sources of fibre. In both cases, the results from this study might contribute to explain observations of decreased diarrhoea mortality when supplementing diets with soluble fibre (Gómez-Conde et *al.*, 2004; Soler *et al.*, 2004; Gidenne *et al.*, 2004; Gidenne and Licois, 2005; Fabre *et al.*, 2006) or when reducing moderately level of insoluble fibre (Gutiérrez *et al.*, 2002).

From the results of this study it can be concluded that in conditions of low incidence of enteritis and/or epizootic rabbit enteropathy, both the decrease of dietary NDF content up to a level of 29-30% and the combined inclusion of soluble fibre and lignin, greatly improved feed efficiency and digestion with little effects on performance.

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