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Response of Growth Performance, Cecal Fermentation Traits and *In vitro* Gas Production to Substitution of Soyhulls for Lignified Fiber in Rabbit Diets*

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ABSTRACT : A growth trial (Expt. 1) and an *in vitro* fermentation experiment (Expt. 2) were conducted to determine the response of growth performance, cecal fermentation characteristics and *in vitro* gas production to incremental levels of substitution of digestible fiber for lignified fiber in the diet of weaned rabbits. Three diets, formulated by substituting soyhulls (SH; used as digestible fiber source) for soybean straw (used as lignified fiber source) at substitution levels of 0, 25 and 50%, were used in a factorial design. In the growth trial (Expt. 1), increasing levels of SH substitution resulted in a quadratic increase in daily body weight gain rate ($p<0.04$) and feed conversion efficiency ($p<0.02$), but in a numerical decrease in dietary DM intake ($p = 0.15$). When SH were included in the diet at 25% substitution level, rabbits had the highest rate of liveweight gain and feed conversion efficiency. As SH substitution level increased, pH values and ammonia-N of cecal contents linearly ($p<0.001$) decreased, but total VFA concentration linearly ($p<0.03$) increased. With incremental levels of SH substitution, the percentage of acetate and butyrate linearly ($p<0.05$) reduced, but the percentage of propionate and minor acids linearly ($p<0.03$) increased. Increasing the SH substitution levels tended to increase incidence of diarrhea. In the *in vitro* fermentation experiment (Expt. 2), regardless of origin of substrates fermented, increasing SH substitution level resulted in increased maximal gas production ($p<0.001$) and shortened gas production lag time, but had no effect on gas production rate ($p>0.2$). These observations suggest that incrementally feeding SH to rabbits could stimulate their cecal microbial activity, allowing cecal fermentation to shift towards favoring fiber digestion. In conclusion, digestible fiber from soyhulls may partially substitute for more lignified fiber, soybean straw, without having an adverse effect on cecal fermentative and microbial activity and growth performance. For growing rabbits, about 73% of total dietary NDF should be supplied by effective NDF, the remainder could come from digestible NDF, such as soyhulls. (**Key Words :** Soyhulls, Weaned Rabbits, Growth Performance, Gas Production, Effective Fiber)

INTRODUCTION

Dietary fiber is important in rabbit nutrition. Rabbits require dietary fiber not only for substrate in cecal microbial fermentation, producing volatile fatty acids and providing microbial proteins to the animal through cecotrophy, but for a scabrous and physical effect on the gut mucosa, maintaining normal gut motility and preventing digestive disturbances (Pond et al., 1995). Local available crop residues, such as soybean straws, are commonly used as a fibrous source for feeding rabbits in the northern part of China. Because these by-products are highly lignified and have mould problems, the animal performance is usually limited. Therefore, exploitation of alternative fibrous feeds becomes quite indispensable.

Soyhulls (SH) are a byproduct of processing soybean for oil and dehulled soybean meal production. Because of their high fiber content, SH are frequently thought to be a good roughage extender. Although high in fiber, SH have higher digestibility of DM and NDF than alfalfa and sunflower hulls (Garcia et al., 2000). Thus, inclusion of soyhulls in the diet of rabbits may induce effects similar to those of low dietary fiber levels on digestive disturbances. Previous researchers (Fraga et al., 1991; Garcia et al., 1999) have demonstrated that the digestible fiber from beet pulp, citrus pulp, soyhulls or rice hulls induced relatively long retention time of digesta in some segments of the gut and thus altered some digestive criteria. These results suggest that highly digestible fiber may not be adequate to meet the fiber requirements of rabbit diets, and the recommended level required must vary with the type of fiber. Because

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Table 1. Ingredients and chemical composition of experimental diets

Item	SH substitution (%)		
	0	25	50
Ingredients (%)			
Cracked corn	25.2	25.5	26.7
Wheat grains	17.0	18.4	17.5
Soybean meal	20.8	19.2	18.7
Soyhulls	0	8.5	17.0
Soybean straw meal	34	25.5	17.0
Limestone	0	0.1	0.2
Bone meal	1.5	1.3	1.4
Mixed premix [a]	1.0	1.0	1.0
Salt	0.5	0.5	0.5
Chemical composition			
DE (MJ/kg DM) [b]	13.4	13.8	14.2
CP (% of DM)	16.7	16.7	16.9
NDF (of DM)	31.6	31.4	31.1
Digestible NDF (of NDF)	17.7	27.6	37.7
ADL (of DM)	5.7	4.7	3.6
Lysine (of DM)	0.84	0.84	0.85
Met+cys (of DM)	0.53	0.53	0.54
Ca (of DM)	0.79	0.74	0.75
P (of DM)	0.52	0.51	0.52

[a] Containing 10,000 mg/kg Fe; 2,400 mg/kg Cu; 8,400 mg/kg Mn; 13,000 mg/kg Zn; 160 mg/kg I; 70 mg/kg Se; 100 mg/kg Co; 960,000 IU/kg VA; 200,000 IU/kg VD_3; 7,500 IU/kg VE.

[b] Calculated from tabular values (NRC, 1977), in which DE content of 16.7, 15.1, 14.9, 15.1 and 10.5 MJ/kg DM were used for cracked corn, wheat grains, soybean meal, soyhulls and soybean straw meal, respectively.

current fiber requirement systems based on total dietary fiber for rabbits are seldom used to express the requirement of a large array of fibrous sources, more information is needed before accurate recommendations are given for various types of fibers.

The objective of the present study was to determine the response of growth performance, cecal fermentation characteristics and *in vitro* gas production on fibrous feeds to substitution of soyhulls at incremental levels for lignified fiber in growing rabbit diets.

MATERIALS AND METHODS

Experimental diets

Three diets were prepared as complete feeds and balanced to meet all the essential nutrient requirements for weaned rabbits according to NRC (1977). Soyhulls (SH) substituted soybean straw in the diet at the level of 0, 25 and 50%. Dietary formulations and chemical composition of the experimental diets are presented in Table 1. Each diet was ground through a 4-mm screen in a hammer mill (Fenglei 9FQ Feed Grinder, Yanjing Animal Husbandry Machinery Group, Beijing) and then pelleted (4 mm in diameter×15-22 mm in length). SH was shipped from Donghai Oil and Grains Industries Co., Ltd. (Zhangjiagang F.T.Z., Jiangsu Province, China). Other ingredients were obtained from locally available sources.

Experiment 1 (Growth trial)

This study was conducted with the approval of the CAU Animal Science and Technology College Animal Care Committee. A total of forty-five weaned rabbits (New Zealand White, 30 males and 15 females, weaned at 30 d of age) were divided into 15 experimental groups with 3 animals (two females and one male) per group. Five groups of rabbits (15 animals) were assigned to each of the three diets in a completely randomized design. Rabbits were housed in groups and maintained in heat-sterilized, wire-floored metal cages (28 cm×62 cm×34 cm) at 18-23°C ambient temperature. The experiment lasted 30 days.

Animals were weighed at the beginning and the end of the trial, while feed consumption was recorded daily. Feed and water were available *ad libitum* to the animals.

Experiment 2 (*In vitro* fermentation experiment)

The mixed microorganisms from cecal contents of the animals in the growth trial were used for *in vitro* fermentation. At the end of the growth trial, three rabbits were randomly selected from each treatment and slaughtered by cervical dislocation. The cecum was immediately exteriorized and cecal contents were removed. The contents from rabbits fed the same ration were pooled and taken to the laboratory for processing within 10 min of collection. A fresh sample (~250 g) was weighed into 250 ml of anaerobic buffer solution (Menke et al., 1979) in 1,000-ml flasks under a constant flow of O_2-free CO_2 and homogenized in a blender under CO_2 for 2 min. After being strained through four layers of cheesecloth, the filtrate was immediately subjected to pH determination with a combination electrode. A portion of the filtrate was sampled and frozen at -20°C for determination of ammonia-N and volatile fatty acid (VFA) content. A solution of 25% metaphosphoric acid was added (5 parts filtrate: 1 part acid ratio) to the filtrate samples for VFA determination. Samples for ammonia-N determination were acidified with a solution of 0.2 M hydrochloric acid (1 ml/ml). The remaining filtrate containing mixed cecal microorganisms was used for making inoculum fluid for the *in vitro* fermentation experiment. The inoculum fluid was prepared by mixing one part of the filtrate with two parts of the artificial saliva medium consisting of *in vitro* anaerobic buffer solution, macro- and micro-mineral solutions, resazurine and reduction solution as described by Menke et al. (1979). Representative samples of soyhulls and soybean straw were obtained from China Agric. Univ. Experimental Rabbit Farm and ground through a 1-mm screen. Each of the fibrous feeds (500 mg DM) was weighed into 6 syringes and incubated with 50 ml of the inoculum fluid at 39±0.2°C

Table 2. Growth performance of weaned rabbit

Item	SH substitution (%)			SEM	Probability [a]	
	0	25	50		L	Q
Rabbit number	15	15	15	-	-	-
Initial weight (g)	1,045.0	1,069.7	1,023.3	-	-	-
Final weight (g)	2,293.0	2,494.8	2,319.2	-	-	-
Daily gain (g)	41.6	47.5	43.2	1.8	0.54	0.04
Dietary DM intake (g/day)	143.5	141.6	136.5	3.2	0.15	0.69
Feed conversion (F/G)	3.47	2.99	3.16	0.10	0.07	0.02

[a] Probability of linear (L), and quadratic (Q) responses.

for 48 h as described by Menke et al. (1979) in a ruminant *in vitro* experiment. Gas production measurements (ml) were taken after 0, 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation and the results, corrected for the blank, were fitted to the one-component exponential model (logistic curves) of Schofield (2000) with a modification, $Y = A\times(1+e^{(2-4\times C\times(t-Lag))})^{-1}$, where Y is *in vitro* gas volume (ml) at time t, A is the asymptotic (theoretically maximal) gas volume (ml), C is the specific rate of gas production per hour (h^{-1}), and Lag is the lag time (h) before gas production began. The constants A, C, and Lag for each fiber source are parameters to be calculated using a non-linear regression procedure (SAS, 1996).

Analytical procedures

Fibrous feed samples were analyzed for DM, Ca and P (AOAC, 1990) and for NDF and acid detergent lignin (Van Soest et al., 1991). Crude protein (CP) content was measured with a Nitrogen Analyzer (Model Rapid N III, Elementar, Analysen Systeme GmbH, Germany) based on the Dumas combustion method (AOAC, 1996). The content of amino acids was analyzed using an automated amino acid analyzer (Model 835-50, Hitachi) following 22 h hydrolysis in 6 N HCL at 110°C for lysine, and oxidization with performic acid overnight according to the AOAC (1990) for sulfur containing amino acids. Frozen cecal filtrate samples were thawed at room temperature and centrifuged at 10,000×g for 10 min. Ammonia concentration of the supernatant was determined spectrophotometrically at 660 nm according to Broderick and Kang (1980). The acidified supernatant samples (0.6-μl portion) were analyzed for VFA by gas chromatography using an Agilent 6890N GC equipped with a 30-m, 0.32-mm i.d. fused Supelcowax TM-10 capillary column (Supelco, Inc., Bellefonte, PA, USA) in split mode (ratio, 1:100). The carrier gas was helium with a flow rate of 50 ml/min and 2-ethylbutyric acid was used as an internal standard. The injector port temperature was 280°C, FID detector temperature was 280°C, and the oven temperature was held at 145°C.

Statistical analysis

Data from the animal growth and *in vitro* fermentation experiments were subjected to analysis as a completely randomized design according to the GLM procedure of SAS (1996). Linear and quadratic responses were also determined using orthogonal contrast statements.

RESULTS

Soyhulls (DM) used in this study contained 12.2% CP, 66.3% NDF, 2.14% acid detergent lignin (ADL), 0.42% Ca, 0.26% P, 0.68% lysine and 0.34% methionine+cystine, whereas soybean straw (DM) contained 6.2% CP, 69.2% NDF, 14.8% ADL, 0.98% Ca, 0.16% P, 0.27% lysine and 0.14% methionine+cystine. Because no data is available for pure SH and soybean straw in the literature, we assumed the NDF digestibility of SH and soybean straw to be 65% and 10%, respectively. This assumption is based on the statement of Cheeke (1987), who noted that generally the digestibility is less than 15% for lignified fiber, but higher than 60% for non-lignified fiber in rabbits. As a result, the contents of digestible NDF increased as SH inclusion increased in the diets. Although substitution of SH for soybean straw increased the content of Ca and CP, and decreased the content of P, these nutrients could be well balanced through adjusting the relative proportion of cracked corn, wheat grains, soybean meal, limestone and bone meal during the diet formulation (Table 1).

Effect of SH substitution of soybean straw on growth performance

The results of rabbit growth performance are shown in Table 2. Increasing SH levels of substitution for soybean straw resulted in a quadratic increase in daily body weight gain rate ($p<0.04$) and feed conversion efficiency ($p<0.02$), but in a numeric decrease in dietary DM intake ($p = 0.15$). When SH were included in the diet at 25% substitution level, rabbits had highest gain rate and feed conversion efficiency (Table 2). Compared with feeding 25% SH substitution diet, feeding no SH and 50% SH substitution diets decreased gain rate by 12.4% ($p = 0.04$) and 9.1% ($p = 0.11$) and decreased feed conversion efficiency by 16.1% ($p = 0.007$) and 5.7% ($p = 0.14$). These results indicate that there might be an optimum level for inclusion of digestible or lignified fibers in rabbit diets for maximizing animal growth performance.

Table 3. Cecal microbial fermentation traits

Item	SH substitution (%)			SEM	Probability [b]	
	0	25	50		L	Q
pH	6.88	6.66	6.45	0.04	0.00	0.72
NH_3-N (mg/100 ml)	8.49	7.09	6.30	0.14	0.00	0.13
TVFA (mM/L)	40.0	41.4	45.3	1.4	0.03	0.49
VFA molar percentage (%)						
Acetate	77.4	74.6	72.9	1.1	0.03	0.73
Propionate	6.8	9.6	13.7	0.7	0.00	0.47
Butyrate	15.2	15.0	12.4	0.8	0.05	0.25
Minor VFA [a]	0.6	0.8	1.0	0.2	0.00	0.07

[a] Isobutyrate+valerate+isovalerate. [b] Probability of linear (L), and quadratic (Q) responses.

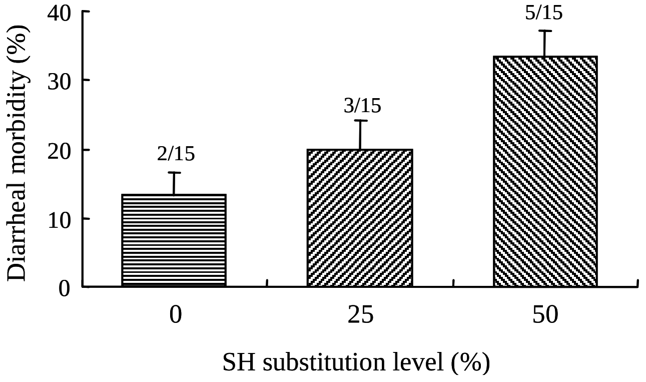

Figure 1. Effect of substitution of soyhulls for soybean straw on diarrhea morbidity.

Effect of substitution of soyhulls for soybean straw on cecal microbial fermentation traits and on diarrhea morbidity

Cecal pH, ammonia-N concentration, total VFA concentration and VFA molar proportions are reported in Table 3. As SH substitution level increased, pH values and ammonia-N of cecal contents linearly ($p<0.001$) decreased, but total VFA concentration linearly ($p<0.03$) increased. The decreased pH values, decreased ammonia-N concentration and increased VFA concentrations showed SH to be highly fermentable in the cecum. As to changes of cecal VFA profile, the percentages of acetate and butyrate linearly ($p<0.05$) reduced, but the percentages of propionate and minor acids linearly ($p<0.03$) increased with incremental levels of SH substitution. The change in cecal VFA profile as a result of inclusion of SH may reflect an alteration in the ecosystem of cecal microflora.

Although death due to feeding the diets with SH did not occur, increasing the SH substitution levels tended to increase incidence of diarrhea (Figure 1). All the digestive disturbance occurring in the animals were found to be transitory (diarrhea during first 2 or 3 days).

Effect of substitution of soyhulls for soybean straw on *in vitro* gas production

With incremental level of SH substitution for soybean straw in the diet, soyhulls and soybean straw used as fermentative substrates exhibited different gas production pattern. Regardless of origin of substrate that was fermented, increasing SH substitution level resulted in increased maximal gas production ($p<0.001$) and shortened gas production lag time, but had no effect on gas production rate ($p>0.2$; Table 4). Increasing SH substitution level linearly ($p<0.001$) increased maximal gas production and reduced gas production lag time for both substrates. These results suggested that incrementally feeding SH to rabbits could stimulate their cecal microbial activity, allowing the fermentation to shift towards favoring fiber digestion. Compared with soybean straw, soyhulls fermented by cecal

Table 4. *In vitro* gas production kinetics of soyhulls and soybean straw incubated with cecal mixed microorganisms from rabbits fed the diets with 0, 25 or 50% SH substitution

Item [c]	SH substitution (%)			SEM	Probability [a]	
	0	25	50		L	Q
Maximal gas (ml/0.5g DM)						
SH	76.2	81.7	92.3	0.4	0.00	0.01
Soybean straw	20.7	24.8	32.2	0.6	0.00	0.07
Gas producing rate (h^{-1})						
SH	0.032	0.033	0.035	0.001	NS [b]	NS
Soybean straw	0.036	0.036	0.036	0.001	NS	NS
Gas producing lag time (h)						
SH	6.1	4.6	4.5	0.3	0.01	0.04
Soybean straw	14.0	10.1	9.7	0.4	0.00	0.03

[a] Probability of linear (L), and quadratic (Q) responses. [b] NS = not statistically significant ($p>0.15$).

[c] Logistic model: $Y = A\times(1+e^{(2-4\times C\times(t - Lag))})^{-1}$

microorganisms from rabbits fed the diets with 0, 25 and 50% of SH substitution had 3.7-, 3.3- and 2.9-fold greater maximal gas production and 2.3-, 2.2- and 2.2-fold shorter gas production lag time, respectively.

DISCUSSION

The results of our study showed reduced feed intake and increased rate of weight gain and feed conversion efficiency with incremental levels of SH substitution for soybean straw in rabbit diets (Table 2). Although fiber content of SH and soybean straw were almost equal, the fiber from SH is highly digestible (Garcia et al., 1999). Therefore, inclusion of SH in rabbit diets would benefit animal growth performance. With incremental inclusion level of SH, however, the daily rate of gain and feed conversion efficiency increased quadratically rather than linearly with the highest performance achieved at 25% SH substitution level. The lowered performance on the SH-free diet may be explained by the lower digestibility of soybean straw due to its highly lignified fiber composition compared with that of SH. On the other hand, the lower performance for the high inclusion SH diet (50% substitution) would be associated with high digestibility of SH in the cecum. Although there has been no evidence showing SH at high inclusion rate in the diet impaired rabbit growth performance, studies using other kinds of digestible fiber (such as beet pulp, citrus pulp and grape marc) could provide indirect confirmation. These studies (Candau et al., 1979; Garcia et al., 1993; Carabano et al., 1997) demonstrated that inclusion of a highly digestible source of fiber in the rabbit diet increased cecal acidity and diarrhea incidence, and thereby impaired growth performance. Furthermore, small particle size of soyhulls used in this study would be a factor influencing rabbit growth performance, as previously observed by Garcia et al. (2000), who found that small particle size of dietary fiber can cause high cecal acidity, more soft feces excretion and long retention time of digesta in the gut. In the current study, we did observe that cecal samples from rabbits fed the 50% SH inclusion diet were more sticky than from animals fed diets with no or low SH inclusion rate. These results clearly indicate that there might be an optimum level for inclusion of highly digestible or lignified fiber in rabbit diets for maximizing animal growth performance.

Fermentation of dietary fiber in rabbits mainly takes place in the cecum, with VFA and gas as main end products. In the present study, increasing SH substitution level linearly decreased pH values and ammonia-N concentrations of cecal contents, but increased total VFA concentrations. The decreased cecal pH, the decreased ammonia-N concentration and the higher VFA concentration suggest high fermentation of SH and active microbial synthesis existed in the cecum. This observation is consistent with the growth performance result discussed above, and is also in agreement with the results of Garcia et al. (2000), who found that a diet with SH as a sole fiber source induced much lower cecal pH and ammonia-N concentration, and a fairly high concentration of cecal VFA when compared between six fibrous sources. The low cecal pH resulting from a high rate of carbohydrate fermentation in the cecum can inhibit normal microbes and allows proliferation of pathogens (Pond et al., 1995), which may in part explain why the incidence of digestive disturbances increased with incremental levels of SH substitution (Figure 1).

As to changes of cecal VFA profile, the percentage of acetate and butyrate linearly reduced, but the percentage of propionate and minor acids linearly increased. It has been established that butyrate plays an important role in maintaining gut epithelial health and the normal turnover of the gut (Roediger, 1980). Whether cecal motility could be impaired by the depressed butyrate production due to SH inclusion is yet unclear. From the present result, cecal microbial fermentation pattern seems to shift to one of high propionate production with incremental substitution of SH. A higher proportion of propionate can result in better feed conversion because of less energy loss owing to less ATP needed by microbes and less heat increment occurring in the rumen (Van Soest, 1994). Moreover, a change in cecal VFA profile as a result of inclusion of SH may also reflect an alteration in the ecosystem of cecal microflora.

In vitro gas (usually CO_2, CH_4 and H_2) production can reflect the extent to which various fibrous feeds are fermented by gut microorganisms (Schofield, 2000). Regardless of the origin of substrates fermented, a linearly increased response of maximal gas production and a linearly reduced response of gas production lag time to incremental inclusion of SH were noted. This result, combined with VFA concentration data (Table 3), strongly suggests a change in microbial ecosystems favoring fiber digestion in the cecum due to feeding rabbits SH inclusion diets. The favorable change includes at least an increase in microbial cellulolytic activities and a decrease in lag time until initiation of fiber fermentation. Although these *in vitro* gas production kinetics are not as representative of the practical feeding situation as those from *in vivo* digestion trials, the measurements *in vitro* had simple and timesaving advantages which permitted differences in the microbial fermentation of fibrous feeds to be detected.

Meeting minimum requirements of dietary fiber is no doubt of importance in maintaining the health status and growth performance of rabbits. However, based on the present results and other studies (Garcia et al., 1993; Gidenne et al., 1998), feeding rabbits on diets with almost equal amounts of NDF from different sources showed different growth performance and distinct cecal fermentative activity. This means that the current fiber system based on total NDF can hardly predict the requirement for dietary fiber, and therefore, it is necessary to have a new concept for expressing the fiber requirement. Because effective utilization of dietary NDF by rabbits depends at least on its chemical composition and physical characteristics (e.g. particle size), we suggest introduction of “effective NDF” for expressing effectiveness of the fiber in rabbit feeding. The effective NDF in the ration of rabbits can be defined as the fiber that could effectively stimulate cecal motility and maintain the health status of the animal. The meaning of this concept is quite similar to that in ruminants (NRC, 1996; NRC, 2001). Based on the above definition, highly digestible fiber sources (e.g. soyhulls and beet pulp) would have low values of effective NDF, whereas highly lignified or poor digestible fiber sources (e.g. soybean straw or wheat straw) would have high values. Using our results, assuming that 32% dietary NDF represents the total NDF requirement for growing rabbits, we calculated that dietary lignified fiber contributing to about 23% of dietary DM or 73% of total dietary NDF could meet the minimum requirement for effective fibers. Once the minimum requirement for effective NDF is met, the remaining NDF requirement could be supplied by digestible fiber such as soyhulls without impairing animal health and growth performance. Although a lot of basic work including development of assessment procedures and establishment of effective fiber requirements is lacking, we believe that establishment of any system based on effective fiber requirement will provide a better way of maximizing rabbit feeding under a wide array of fibrous feeds.

IMPLICATIONS

Our results indicate that digestible fiber from soyhulls may partially substitute for more lignified fiber, soybean straw, without an adverse effect on cecal fermentative and microbial activity and growth performance of rabbits. A high level of soyhull substitution seemed to impair the health status and growth performance of the growing rabbit. A new concept, effective NDF, which is defined as the fiber that could effectively stimulate cecal motility and maintain the health status of the animal, was introduced. In order to maximize the animal growth performance with no adverse effect on health status, the minimum requirement of dietary effective NDF must be firstly met. For growing rabbits, about 73% of total dietary NDF should be supplied by effective NDF, the remainder could come from digestible NDF, such as soyhulls.

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