

Responses in Milk Yield, Milk Composition and Rumen Fermentation in Lactating Cows Receiving a Corn Straw or Mixed Forage Diet

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Abstract: An experiment was conducted to investigate responses in milk production, milk composition and rumen fermentation of dairy cows receiving a corn straw or mixed forage diet. Ten primiparous and rumen-cannulated Holstein dairy cows averaging 127±13 days in milk (mean±SD) were randomly assigned to CS (37.1% corn straw as sole forage) or MF diet (3.7% Chinese wildrye+28.4% alfalfa hay+26.5% corn silage as mixed forage). Feed intake, body weight, body condition score and milk production were monitored. Milk fat, protein, lactose and total solids were analyzed by near-infrared analysis. Samples of rumen fluid were collected via cannula every 6 h over a 72 h duration to analyze pH, ammonia-N and VFA concentrations. Results showed that cows fed MF diet significantly increased dry matter intake ($p<0.01$) as well as yields of milk ($p<0.05$), 4% FCM ($p<0.01$), milk fat ($p<0.05$), lactose ($p<0.05$) and total solids ($p<0.05$). Cows fed MF diet had higher ($p<0.01$) ruminal acetate concentration and ratio of acetate to propionate and lower concentrations of ammonia-N ($p<0.01$), propionate ($p<0.05$), butyrate ($p<0.01$), isobutyrate ($p<0.01$), valerate ($p<0.01$) and isovalerate ($p<0.01$) than cows fed CS diet. Collectively, these results suggest that cows fed MF diet improve DMI and milk production. In addition, cows fed CS diet had minor differences in rumen pH and total VFA concentration which may suggest that corn straw played an important role in maintaining rumen function when cows were fed this higher concentrate diet.

Key words: Forage quality, milk production, milk composition, rumen fermentation, China

INTRODUCTION

It has been known for many years that various dietary factors such as forage to concentrate ratio and forage types (Murphy *et al.*, 2000) can affect rumen fermentation and consequently milk production and composition. According to Anonymous (2010) for 2009 the average milk fat and protein contents of China cattle milk were 3.1 and 2.8%, respectively which were far below the levels of United States (3.5 and 3.1%, respectively) and New Zealand (4.5 and 3.7%, respectively). The most striking difference between those results is forage quality. In China, crop straw resources are very plentiful, producing >700 million tons of crop straw in a year. The main crop straw is corn straw, accounting for >30% of total crop straw. Corn straws are widely used as main roughage sources for most dairy farms in China. A major constraint of corn straw for livestock production is poor nutrient quality. Hence, ruminants consuming low quality

corn straw sometimes are given intensive concentrate. However, in the United States alfalfa is used as a basic component in feeding programs for dairy cows. Known as Queen of Forages, alfalfa provides highly nutritious forage in terms of protein, vitamins and minerals for ruminant animals.

In addition, a survey carried out in Heilongjiang and Liaoning province in the second half year of 2006 in China showed that compared with cows fed corn straw as sole forage, rolling herd average milk yield (kg), milk fat percentage (%) and milk true protein content (%) of cows fed alfalfa, Chinese wildrye and corn silage as mixed forage were improved by 52.9, 19.0 and 7.9%, respectively (Yu, 2007).

This study was designed to identify if significant differences in milk production and composition were due to differences in rumen metabolism of different forage quality diets as a basis for explaining why milk fat and protein levels in China are lower compared with United States.

MATERIALS AND METHODS

Management of animals: Ten primiparous, lactating Holstein cows (127±13 days, Day in Milk (DIM)±SD) fitted with rumen cannulae were used in this experiment. Duration of experiment was 9 weeks with a 2 weeks adaptation period. Throughout the experimental period, cows were housed in a tie-stall barn and fed individually and water was available *ad libitum*. They were milked at 0430 and 1530 h and fed a TMR twice a day at 0700 and 1900 h. Daily intakes of feed were assessed during the 2 weeks adaptation period and these daily intakes were re-evaluated during experimental period to enable cows to refuse at least 500 g DM/kg DM of feed offered. Animal care and procedures were approved and conducted within standards of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Diets: Diets were formulated to meet nutrient requirements for a 550 kg mature cow producing 20 kg day⁻¹ of milk containing 3.5% of milk protein and 4.5% of milk fat according to the Feeding Standards of Dairy Cattle, China NongYe HangYe BiaoZhun/Tuijian-34 (China NY/t34, 2004). Treatments were: Corn Straw (CS): 37.1% of corn straw as the only roughage and 62.9% of concentrate, Mixed Forage (MF): 58.6% of mixed forage (3.7% Chinese wildrye + 28.4% alfalfa hay + 26.5% corn silage) and 41.4% of concentrate. Ingredients and chemical composition of diets used in this study are presented in Table 1. Increasing the proportion of concentrate mixture in CS diet increased the dietary crude protein from 16.67-18.48%. The CS and MF diets had forage to concentrate ratios of 40:60 and 60:40, respectively.

Sample collection and analysis: Feed intakes and orts were recorded daily. Samples of TMR and orts were collected twice a week and frozen at -20°C for DM analysis at 65°C for 48 h. Dried dietary ingredients were ground to pass through a 1 mm screen using a lab mill (ZM200, Retsch, Haan, Germany) and analyzed for concentrations of CP, Ether Extract (EE), calcium, phosphorus (AOAC, 2000), NDF and ADF (Van Soest *et al.*, 1991). Net Energy of lactation (NE_L) was determined based on chemical composition and 24 h gas production of TMR (Menke and Steingass, 1988). Dry matter concentration was determined after drying samples at 105°C for 6 h and all chemical analyses were expressed on DM basis.

Body Weight (BW) and Body Condition Score (BCS) were determined at beginning and end of experiment. Cows were weighed before morning feeding. BCS was

Table 1: Ingredient and nutrient composition of Corn Straw (CS) and Mixed Forage (MF) diets

Items	Treatment	
	CS	MF
Ingredient (g/100 g of DM)		
Chinese wildrye	-	3.70
Corn straw	37.10	-
Alfalfa hay	-	28.40
Corn silage	-	26.50
Concentrate mixture ¹	62.90	41.40
Nutrient composition (g/100 g of DM)		
DM	54.47	55.78
NE _L (Mcal/kg of DM ²)	1.48	1.52
CP	18.48	16.67
NDF	41.01	44.18
ADF	21.15	26.06
EE	1.58	2.24
Ca	0.89	0.82
P	0.21	0.31

¹Concentrate mixture was composed of corn, soybean meal, wheat bran, cottonseed, calcium hydrophosphate, limestone, NaCl, trace mineral and vitamin premix (vitamin A, vitamin D and vitamin E); ²NE_L = Net Energy for Lactation, determined based on chemical composition and 24 h gas production of feeds (Menke and Steingass, 1988)

obtained using a 5 point system described by Wildman *et al.* (1982). Milk yield of each cow was recorded at each milking. Milk samples from a.m. and p.m. milkings were taken twice a week and stored at 4°C with a preservative until analyzed for fat, protein, lactose and total solid by near-infrared analysis using the Milk-O-Scan 133B (Foss Electric, Hillerod, Denmark).

Samples of ruminal fluid were collected via cannula every 6 h over a 72 h duration starting on day 37 of experiment period to account for diurnal variation. Subsamples of rumen fluid were collected in equal proportions from cranial dorsal, cranial ventral, caudal dorsal, caudal ventral and central rumen regions and combined to form one sample. Rumen pH was measured immediately at each sampling time using a SevenGo™ pH-SG2 PH meter (METTLER TOLEDO, Shanghai, China). Rumen fluid samples were strained through four layers of cheese cloth and frozen at -20°C until further analysis. Ammonia nitrogen concentration of rumen fluid samples was determined using method described by Broderick and Kang (1980). Concentrations of Volatile Fatty Acid (VFA) were quantified by gas chromatography (Agilent 6890N) with a 15 m capillary column (0.32 mm i.d., Agilent DB-FFAP) and a flame ionization detector.

Statistical analysis: Data were analyzed using MIXED Models of SAS Institute (2003) (Version 9.0, SAS Institute Inc., Cary, NC) with repeated observations. Fixed effects included treatment, sampling time and treatment x time interaction. Cow was considered as random variable. Significance level was declared at p<0.05. Trends for significance were declared at p = 0.05-0.10.

RESULTS AND DISCUSSION

Feed intake, BW and BCS: As shown in Table 2, there were no significant differences in BW and BCS between the two dietary treatments ($p>0.05$). Cows fed MF diet had higher Dry Matter Intake (DMI) than cows fed CS diet (20.4 vs. 18.5 kg day⁻¹; $p<0.01$).

Milk yield and composition: Milk production data are presented in Table 2. Cows fed CS diet produced less milk ($p<0.05$), 4% fat corrected milk (FCM, $p<0.01$), fat ($p<0.05$), lactose ($p<0.05$) and total solids ($p<0.05$) by 12, 15, 16, 12 and 12%, respectively, compared with cows fed MF diet. Milk protein yields (kg/day) as well as proportions of milk fat, protein, lactose and total solids (%) did not differ between the two dietary treatments ($p>0.05$).

Rumen fermentation parameters: Relative to MF treatment, the CS treatment had increased ($p<0.01$) concentration of ammonia-N (Table 3). There were no treatment effects on mean rumen pH and total VFA concentrations ($p>0.05$). Feeding CS diet decreased ($p<0.01$) molar proportion of acetate and acetate to propionate ratio by 7 and 17%, respectively and increased

molar proportions of propionate ($p<0.05$), butyrate ($p<0.01$), isobutyrate ($p<0.01$), valerate ($p<0.01$) and isovalerate ($p<0.01$) by 11, 18, 33, 16 and 26%, respectively. Time of sampling significantly ($p<0.01$) altered rumen pH, ammonia concentrations, total VFA concentrations, molar proportions of acetate, propionate and butyrate as well as the acetate: propionate ratio.

Postprandial changes in rumen pH, ammonia concentrations and molar concentrations of acetate, propionate and butyrate are presented in Fig. 1a-e. Following morning feeding (0700 h) rumen pH for both treatments sharply decreased within first 3.5 h and then showed a gradual increase up to near pre-feeding level. Time between evening feeding (1900 h) and rumen pH nadir was longer with CS treatment. At 0830, 1230 and 2230 h rumen pH was significantly higher for CS treatment relative to MF treatment ($p<0.05$). Rumen ammonia-N concentrations in both diets peaked at 1030 h during diurnal samplings and peaked at 2030 h during nocturnal samplings. Lowest rumen ammonia-N concentrations for both diets were observed at 1630 h. Among sampling times, rumen ammonia concentrations in CS treatment were all observed significantly higher than MF treatment ($p<0.05$). Changes in pattern of acetate, propionate and butyrate over sampling times were similar. In both treatments VFA concentrations peaked at approximately 1.5 h after morning feeding (Fig. 1c-e). Time between evening feeding and maximum VFA concentrations was longer with CS treatment.

DMI: Effects of forage source and level on DMI in ruminants have been studied before (Galyean and Defoor, 2003). Based on their results, percentage of neutral detergent fiber supplied by forage in diets can be used to predict effects of forage source and level on DMI in feedlot cattle with very different rations. Biological reasons for changes in DMI associated with changes in roughage source and level are not understood fully. Most available literature points to digestibility and rate of ingesta passage and physical fill as primary mechanisms of intake regulation in ruminants (Waldo, 1986; Allen, 1996).

Alfalfa hay is a legume that serves as a source of protein and fiber in dairy cattle rations. According to survey of Mowrey and Spain (1999), about 62% of dairy cows relied on alfalfa hay in the United States. As alfalfa hay inclusion rate in diets increased from 0-21%, DMI of dairy cattle tended to increase linearly (Mullins *et al.*, 2009). Improvement in intakes of low quality barley straw as a result of supplementation with alfalfa hay has also been reported in beef diets by Preston and Parra (1981). Higher DMI observed in this study for MF treatment was

Table 2: Effect of dietary treatments on BW, BCS, DMI, milk yield and composition of milk components

Items	Treatment		SEM	p-value
	CS	MF		
BW (kg)	523.00	547.00	9.750	0.1100
Body Condition Score (BCS)	2.78	2.88	0.063	0.3100
DMI (kg/day)	18.50	20.40	0.360	0.0063
Yield (kg/day)				
Milk	18.70	21.20	0.570	0.0150
4% FCM	20.50	24.10	0.710	0.0079
Fat	0.87	1.04	0.039	0.0150
Protein	0.76	0.81	0.025	0.1700
Lactose	0.88	1.00	0.031	0.0230
Total solid	2.67	3.04	0.084	0.0150
Composition (%)				
Fat	4.69	4.91	0.210	0.4900
Protein	4.05	3.82	0.073	0.4600
Lactose	4.68	4.72	0.031	0.4300
Total solid	14.33	14.33	0.260	0.9900

Table 3: Effects of dietary treatments on rumen fermentation parameters

Items	Treatment		SEM	p-value		
	CS	MF		Trt	Time	Trt x time
Rumen pH	6.12	6.01	0.045	0.1400	<0.0001	0.1600
Ammonia (mg/dL)	36.45	23.02	1.320	<0.0001	<0.0001	0.0007
Rumen VFA						
Total VFA (mM)	106.80	107.10	1.830	0.9100	<0.0001	0.0360
Acetate (%)	65.80	70.70	0.540	0.0002	<0.0001	0.0091
Propionate (%)	23.00	20.50	0.550	0.0130	<0.0001	<0.0001
Butyrate (%)	6.00	4.90	0.130	0.0003	<0.0001	0.0069
Isobutyrate (%)	0.60	0.40	0.015	<0.0001	<0.0001	0.1900
Valerate (%)	1.90	1.60	0.037	0.0001	<0.0001	0.2200
Isovalerate (%)	2.70	2.00	0.055	<0.0001	<0.0001	0.1700
Acetate: propionate	2.88	3.47	0.100	0.0041	<0.0001	0.0005

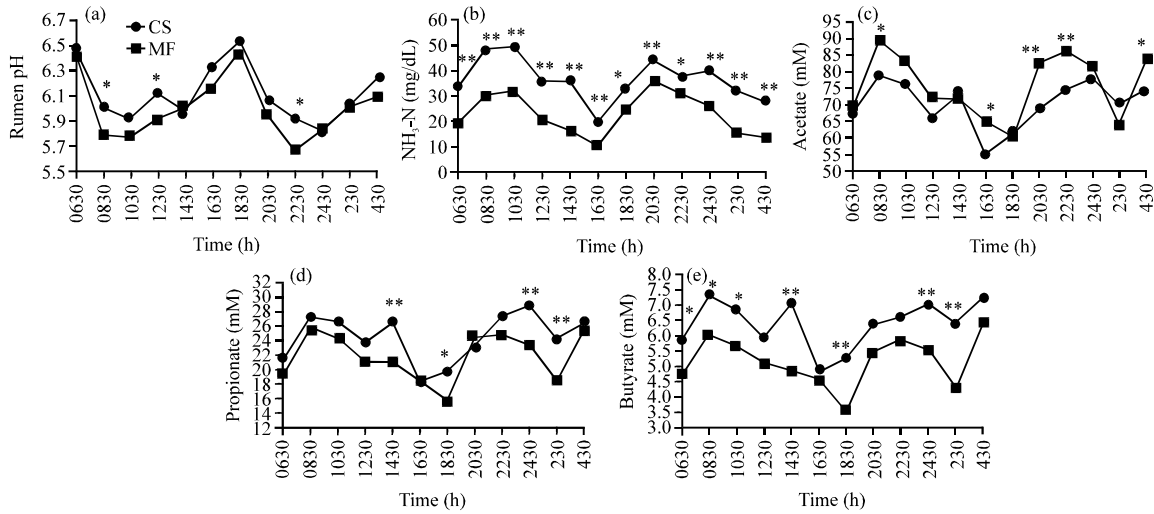


Fig. 1: Effects of dietary treatments on; a) rumen pH; b) rumen ammonia N (mg/dL); c) acetate (mM); d) propionate (mM); e) butyrate (mM). * $p < 0.05$; ** $p < 0.01$

probably a result of a lower filling effect of the alfalfa hay due to a faster rate of digestion and passage from the rumen (Jung and Allen, 1995).

Rumen fermentation: In this experiment an unexpectedly higher mean rumen pH was observed in CS treatment compared with MF treatment (6.12 vs. 6.01; Table 3) as CS diet contained more concentrate. Part of the reason for this might intrinsic buffering capacity of dietary fiber which affects rumen buffering. Corn silage has a lower intrinsic buffering capacity than alfalfa silage (McBurney *et al.*, 1983), facilitating a lower rumen pH. Moreover, Haaland *et al.* (1982) showed that ruminal ammonia could neutralize about 10-15% of total VFA production and higher ruminal ammonia concentrations are often associated with a higher ruminal pH and ruminal buffering capacity. In the experiment, cows fed CS diet produced 37% more ammonia than that of MF treatment which may explain unanticipated results. This also indicated that moderate levels of corn straw may be beneficial for ruminal function in high concentrate fed dairy cows. More research is required to understand mechanisms of rumen microorganisms' adaptation to corn straw with a high concentrate diet.

The pH nadir occurred at time 3.5 h after morning feeding which was comparable with result found by Zebeli *et al.* (2008). Time between evening feeding (1900 h) and rumen pH nadir was longer with CS treatment which reflected that low rumen pH may have been affected more by forage fermentation rather than concentrate fermentation (Van Vuuren *et al.*, 1986).

Rumen ammonia-N concentration peaked in the morning when pH was at its lowest level which was probably the result of considerable eating activity after the morning feeding. Cows fed CS diet had a higher ruminal concentration of ammonia than those fed MF diet (36.45 vs. 23.02 mg dL⁻¹; Table 3) which was due to a higher diet protein content and substantial degradation of diet protein in the rumen. Reduced ammonia-N concentration in cows fed MF diet may indicate a higher microbial efficiency by rumen microorganisms related to increased fractional passage rates that usually occur upon higher DMI levels (Dijkstra *et al.*, 2002; Suarez *et al.*, 2007).

Time between evening feeding (1900 h) and maximum VFA concentrations was longer with CS treatment which was also consistent with pH results across sampling times. Ruminal volatile fatty acid accumulation could cause ruminal pH to decline. Ruminant diets based on roughage, especially those rich in structural carbohydrates are known to produce high molar proportion of acetate and consequently increase acetate to propionate ratio (Barnink *et al.*, 2006). In line with those observations, cows fed MF diet containing more fiber produced more concentration of ruminal acetate in this study. Also, molar proportions of propionate and butyrate were higher in cows fed higher concentrate CS diet. However, rumen ecologies were generally similar between dietary treatments because there were not significant differences in mean value of rumen pH and total VFA concentration.

Milk yield and composition: Changes in milk fat yield with changes in forage type in this study can be explained by

changes in rumen fermentation patterns. Increased milk fat content and yield in MF diet was directly related to higher acetate content and ruminal acetate: propionate ratio. Acetic acid is the principal substrate for lipogenesis and propionic acid is the only glucogenic VFA. Response in milk yield may have been due to significant difference in DMI between two dietary treatments. Martin and Sauvant (2002) reported a relatively high correlation between DMI and milk yield in mid and late lactation cows, suggesting importance of DMI as a driving force in milk production. High feed intake is essential for dairy cows achieving high production and maintaining their prolificacy.

CONCLUSION

These results showed that cows fed MF diet improved DMI and milk production. Reduced ammonia-N concentration in cows fed MF diet may indicate a higher microbial efficiency by rumen microorganisms. In addition, Cows fed CS diet had minor changes in rumen ecologies which may suggest that corn straw played an important role in maintaining rumen function when cows were fed a high concentrate diet.

ACKNOWLEDGEMENTS

Researchers gratefully thank the staff of the Ruminant Nutrition Laboratory, Institute of Animal Science, Chinese Academy of Agricultural Science for their assistance. This research was financially supported by National Key Basic Research Program of China (2011CB100805). Ministry of Science and Technology of China (2012BAD12B02) and State Key Laboratory of Animal Nutrition (2004DA125184G1103) helped researchers in this research.

REFERENCES

AOAC, 2000. Official Method of Analysis of the Association of Official Analytical Chemists. 17th Edn., AOAC International, Gaithersburg, MD., USA.

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. *J. Anim. Sci.*, 74: 3063-3075.

Anonymous, 2010. China dairy statistical report, 2010. Department of China Dairy Yearbook, China Ministry of Agriculture, Beijing, China.

Bannink, A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A.M. Van Vuuren and S. Tamming, 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *J. Theor. Biol.*, 238: 36-51.

Broderick, G.A. and J.H. Kang, 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Dairy Sci.*, 63: 64-75.

China NY/t34, 2004. Feeding Standard of Dairy Cattle. China Nong Ye Hang Ye Biao Zhun/Tuijian-34, China Agricultural Publisher, Beijing, China.

Dijkstra, J., J.A.N. Mills and J. France, 2002. The role of dynamic modelling in understanding the microbial contribution to rumen function. *Nutr. Res. Rev.*, 15: 67-90.

Galyean, M.L. and P.J. Defoor, 2003. Effects of roughage source and level on intake by feedlot cattle. *J. Anim. Sci.*, 81: E8-E16.

Haaland, G.L., H.F. Tyrrell, P.W. Moe and W.E. Wheeler, 1982. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on Rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentration of cattle. *J. Anim. Sci.*, 55: 943-950.

Jung, H.G. and M.S. Allen, 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *J. Anim. Sci.*, 73: 2774-2790.

Martin, O. and D. Sauvant, 2002. Meta-analysis of input/output kinetics in lactating dairy cows. *J. Dairy Sci.*, 85: 3363-3381.

McBurney, M.I., P.J. Van Soest and L.E. Chase, 1983. Cation exchange capacity and buffering capacity of neutral-detergent fibers. *J. Sci. Food Agric.*, 34: 910-916.

Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.*, 28: 7-55.

Mowrey, A. and J.N. Spain, 1999. Results of a nationwide survey to determine feedstuffs fed to lactating dairy cows. *J. Dairy Sci.*, 82: 445-451.

Mullins, C.R., K.N. Grigsby and B.J. Bradford, 2009. Effects of alfalfa hay inclusion rate on productivity of lactating dairy cattle fed wet corn gluten feed-based diets. *J. Dairy Sci.*, 92: 3510-3516.

Murphy, M., M. Akerlind and K. Holtenius, 2000. Rumen fermentation in lactating cows selected for milk fat content fed two forage to concentrate ratios with hay or silage. *J. Dairy Sci.*, 83: 756-764.

Preston, T.R. and R. Parra, 1981. Utilization of tropical crop residues and agroindustrial by-products in animal nutrition: Constraints and perspectives. Proceedings of the FAO/ILCA Workshop, September 21-25, 1981, Dakar, Senegal.

SAS Institute, 2003. The SAS System for Windows. Version 9.0, SAS Institute Inc., Cary, NC., USA.

- Suarez, B.J., C.G. van Reenen, N. Stockhofe, J. Dijkstra and W.J.J. Gerrits, 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. *J. Dairy Sci.*, 90: 2390-2403.
- Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583-3597.
- Van Vuuren, A.M., C.J. van der Koelen and J. Vroons-de Bruin, 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. *Neth. J. Agric. Sci.*, 34: 457-467.
- Waldo, D.R., 1986. Effect of forage quality on intake and forage-concentrate interactions. *J. Dairy Sci.*, 69: 617-631.
- Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt and T.N. Lesch, 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.*, 65: 495-501.
- Yu, Z., 2007. Application of CNCPS to ration evaluation and performance prediction in dairy cattle. Master's Thesis, Northeast Agricultural University, Harbin, Heilongjiang, China.
- Zebeli, Q., M. Tafaj, I. Weber, H. Steingass and W. Drochner, 2008. Effects of dietary forage particle size and concentrate level on fermentation profile, *in vitro* degradation characteristics and concentration of liquid- or solid-associated bacterial mass in the rumen of dairy cows. *Anim. Feed Sci. Technol.*, 140: 307-325.