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The metabolism of linseed lignans in rumen and its impact on ruminal metabolism in male goats

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ABSTRACT

To investigate the metabolism of linseed lignans in the rumen and its impact on ruminal metabolism, four healthy male Huainan goats were used in a crossover study. Following secoisolariciresinol diglucoside (SDG) crude extract treatment, the concentrations of SDG, enterodiol (END) and enterolactone (ENL) in both ruminal fluid and serum were significantly increased ($P<0.01$). Concomitantly, the pH value and ammonia-nitrogen concentration of ruminal fluid were decreased ($P<0.05$), and the concentrations of microbial crude protein and total volatile fatty acids were increased ($P<0.01$). Furthermore, testosterone and 3, 3′, 5-triiodothyronine levels in serum and ruminal fluid were both significantly increased, while the concentration of E_2 in ruminal fluid was decreased. Based on these observations, it is suggested that ruminal microorganisms were able to efficiently convert SDG to END and ENL, which were absorbed immediately. In return, SDG and/or its metabolites may facilitate the utilization of non-protein nitrogen and carbohydrates in the rumen.

KEY WORDS: linseed lignans, mammalian lignans, ruminal metabolism, enterolactone, enterodiol, thyroid hormones

INTRODUCTION

Phytoestrogens are oestrogen-like compounds of plants origin that comprise essentially 2 families-the isoflavones and the lignans. Lignans, which are ubiquitous in many edible plants, are the main source of dietary phytoestrogen.

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Dietary lignans, particularly secoisolariciresinol diglucoside (SDG), are of interest because they have been proposed to play a role in the prevention of breast and colon cancer, atherosclerosis and diabetes (Wang, 2002). Previous studies in non-ruminant animals have indicated that the plant lignans per se are devoid of any biological properties, and that only the mammalian lignans enterodiol (END) and enterolactone (ENL), produced from SDG by intestinal bacteria, have interesting biological properties such as oestrogen agonism and antagonism as well as antioxidative and enzyme-inhibiting activities (Clavel et al., 2005). Earlier studies in rats and humans had established that SDG could be metabolized to END, probably through hydrolysis, dehydroxylation and then demethylation by facultative bacteria, and then ENL was produced from END through oxidation by faculatative bacteria (Wang, 2002); however the bacterial convertation of SDG in rumen have not been reported.

As reviewed by Han et al. (2006), injection of daidzein, one of the main types of isoflavone, *via* duodenal cannulae increased serum testosterone, rumen bacterial protein, ammonia nitrogen and total volatile fatty acids (TVFA). Previous studies have suggested that the absorbed daidzein may facilitate the growth of male animals *via* the neuroendocrine pathway. However, the absorption of lignans in ruminants and the impact of lignans on ruminal metabolism remain unclear. Since both isoflavones and lignans have, as their common denominator, a phenolic group that they share with oestrogenic steroids, it was presumed that lignans may have approximate physiological effect on ruminal metabolism.

Therefore, the aims of the current study were to elucidate the metabolism and absorption of SDG in ruminants and the possible impact of SDG and/or its metabolites (END and ENL) on ruminal metabolism.

MATERIAL AND METHODS

Animals and management

Male Huainan goats (4 animals, fitted with permanent rumen fistulae and temporary catheters in the jugular vein) were housed in well-ventilated, cement-floored individual pens and maintained under conditions of strict hygiene and uniform management. The animals were fed twice per day (08.00 and 18.00 h) at a maintenance energy level [55 g dry matter (DM) per kg body weight (BW) per day] on a basal diet (BD) consisting of 700 $g \cdot kg^{-1}$ hay, and 300 $g \cdot kg^{-1}$ cracked maize (DM basis). In order to control the interference of the other kinds of phytoestrogens, the hay was supplemented without forage legumes, which may contain high level of isoflavones. The animals also had free access to fresh water.

Experimental design and sampling

The goats were randomly assigned to 2 groups of 2 animals each on the basis of BW (20 ± 2.5 kg), in a crossover study with 2 periods (control or treatment period). Each period was of 4 weeks' duration, comprising 14 d of adaptation to the SDG crude extract (SDGCE) or 14 d of washout, followed by 14 d of sampling. During the treatment period, using a cylinder, 50 mg·kg body weight^{-1} SDGCE (20% SDG, w:w; LinumLife, Taiwan) was infused into the rumen of the goats *via* the fistulae when the BD diet was offered.

On days 14, 21, and 28, samples of ruminal contents obtained *via* ruminal cannula and 2 ml blood (collected through a catheter in the jugular vein) were obtained from each goat at 7.00 a.m., and thereafter at 2-h intervals over the next 22 h. The blood samples were centrifuged (1000 *g*, 15 min) in order to obtain sera, which was stored at -20°C until analysis. Ruminal fluid pH and the total activity of dehydrogenase (TDHA) were determined from fresh ruminal samples. Other rumen samples were strained through 4 layers of cheesecloth. Two ml ruminal samples were used for microbial crude protein (MCP) analyses. A 10-ml aliquot of the ruminal fluid was acidified with 1 ml of 6N HCl, and stored in a freezer (-20°C) for ammonia-nitrogen (NH_3-N) analysis. Two millilitres of freshly prepared 25% (250 ml·l^{-1}) metaphosphoric acid was added to 8 ml of strained ruminal fluid. Samples were then centrifuged (17,000 *g*, 10 min), and the supernatant fluid was stored at -20°C for volatile fatty acids (VFA) analysis. And another 2 ml of ruminal fulid was centrifuged (10,000 *g* for 10 min), the supernatant was stored at -20°C until hormone determination.

Chemical analysis

In this study, the levels of mammalian lignans in the serum and ruminal fluid of the 4 male goats were used to assess the bacterial activation and absorption of SDG. The concentrations of SDG, END and ENL in both ruminal fluid and serum were measured by high-performance liquid chromatography (HPLC) (Agilent 1100 Series; Agilent, USA) using the respective authentic standards [SDG (Chromadex, USA); END and ENL (Sigma, USA)] as described by Morton et al. (1997). As Adlercreutz et al. (1995) found that 92% of END and 98% ENL exist in glucuronide conjugates in serum, the analytical procedure for END and ENL in serum included a hydrolysis step using NaOH (1 mol·l^{-1}, 20°C, 48 h).

TDHA may reflect the activities of ruminal microorganisms. In this study, TDHA was analysed following the method described by Dror et al. (1969). Briefly, 4.5 ml rumen liquor and 0.5 ml of triphenyl tetrazolium chloride (TTC, 1.5%, w:w) were incubated at 38° for 5 min. The reaction was stopped by 4.5 ml isopropanol (50%, v:v). After centrifuged, chromatometry were used to determine the total dehydrogenase activity. To examine the effect of SDGCE on

ruminal metabolism, some metabolism parameters, such as MCP, in rumianl fluid were analysed. NH_3-N was measured by the indophenol method (Weatherburn, 1967). The VFA (acetate, propionate and butyrate) were determined using a gas chromatograph (GC-9APTF; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and a capillary column (HP-INNOWAX, 1909N-133; Hewlett-Packard, USA). The temperatures of the detector and column were 220 and 170°C, respectively. Nitrogen was used as a carrier, and total flow and column flow were both 63.8 $ml \cdot min^{-1}$. MCP was determined by the method of Zinn and Owens (1986), based on purine, and estimated from the ratio of purines to nitrogen in isolated microbes.

Hormones determination

The concentrations of testosterone (T); estradiol (E_2); 3, 3′, 5-triiodothyronine (T_3); and thyroxine (T_4) in the serum and ruminal fluid were measured by radioimmunoassay (RIA) using commercial kits purchased from the North Institute of Biological Technology (Beijing, China). This method was based on the binding of antibody (in agent) and specified hormones (the antigen) in samples. These binding have high degree of specificity, for examples: the cross reaction rate of the antibody of E_2 to trihydroxyestrin (E_3), progesterone (P) and testosterone (T) were 0.016%, <0.01 and 0.01%, respectively.

For ruminal samples, a 100-µl aliquot of each ruminal fluid sample were mixed in a tube with activated charcoal (10 ml : 1 g), homogenized, incubated for 3 h with continuous shaking at ambient temperature, and then centrifuged for 10 min (10000 rpm, 4°C). The supernatant was used to correct for the non-specific adsorption of ruminal fluid. The inter- and intra-assay coefficients of variation of the 4 kits were 10 and 15%, respectively. The binding rates of SDG, END, and ENL to E_2 and T antibodies were also determined in the same manner using their respective authentic standards. The antibodies were demonstrated to be specific for T or E_2 and did not cross-react with SDG or the 2 mammalian lignans (END and ENL).

Statistical analyses

For metabolism parameter and hormones concentrations, such as the MCP and T levels, in order to control the influences of other factors (circadian, the sampling time, feed, water intake, and so on), all of the samples were analysed separately, and then the mean levels of each metabolism parameter were calculated on each sampling day (14, 21 and 28-d) for each goat. Based on random factor (goat) and fixed factor (treatment and period), the differences between BD and SDGCE treatment were tested for significance using the analysis of variance by SPSS system (V 11.5, SPSS Inc.) The concentrations of SDG, END and ENL in ruminal

fluid and serum were analysed by the analysis of variance for repeated measures. Data are expressed as means ± standard error (S.E.).

RESULTS

The lignan concentrations in ruminal fluid and serum

There were low levels of END (0.018±0.018 g·l^{-1}) and ENL (0.024±0.021 g·l^{-1}) in ruminal fluid in the ruminal fluid under the basal diet condition (Figure 1). This may indicate that ruminal microorganisms efficiently converted SDG to mammalian lignans; however, those of SDG, END and ENL in the serum were under the detected level.

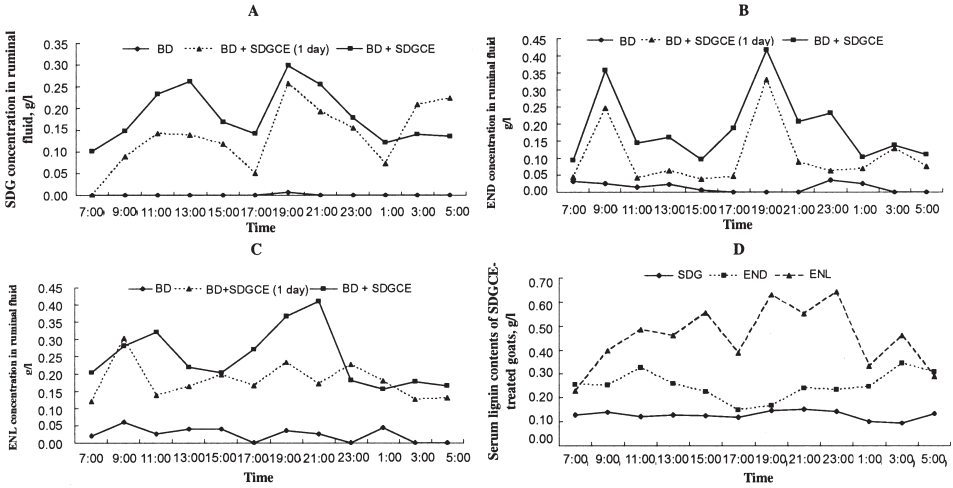

Figure 1. The concentrations of lignans in ruminal fluid and serum in male goats. A, B, and C - secoisolariciresinol diglucoside (SDG), enterodiol (END) and enterolactone (ENL) concentrations, respectively, in the ruminal fluid of goats fed a basal diet (BD) or BD + SDG crude extract (SDGCE). D - serum levels of SDG, END and ENL in goats with SDGCE treatment

As shown in Figure 1-B and C, on the first day when SDGCE was offered, the concentrations of END and ENL (09.00 a.m.) were remarkably increased. The levels of SDG, END and ENL in both ruminal fluid and serum were increased by SDGCE treatment, and their average levels in serum during the treatment period were 0.128±0.018 g·l^{-1}, 0.246±0.068 g·l^{-1} and 0.451±0.130 g·l^{-1}, respectively.

Table 1 summarizes the impacts of SDGCE on ruminal metabolism. Following SDGCE treatment, the pH value of ruminal fluid was significantly decreased by 0.15; however, there was no marked change in the TDHA. In contrast, the

TVFA level (P<0.01) and the (acetate + butyrate): propionate ratio [(A+B): P ratio, P<0.05] were significantly increased by SDGCE. A positive effect on rumen bacterial protein synthesis was also observed; the NH_3-N concentration was remarkably reduced (P<0.05), whereas the MCP level in ruminal fluid was significantly increased by SDGCE (P<0.01).

Table 1. Impacts of secoisolariciresinol diglucoside crude extract (SDGCE) on ruminal metabolism

Item	BD [1]	BD+SDGCE [2]	S.E.	P-value [3]
pH value	6.33	6.18	0.03	0.047
TDHA, $(H^+ \cdot min^{-1}) \cdot l^{-1}$	7.38	12.07	1.60	0.843
TVFA, $mmol \cdot l^{-1}$	15.73	23.25	1.43	<0.001
Acetate, A, mol·%	50.17	52.10	0.44	0.129
Propionate, P, mol·%	31.45	26.34	0.31	0.021
Butyrate, B, mol·%	18.38	21.55	0.15	0.062
(A+B): P ratio	2.18	2.80	0.09	0.044
NH_3-N, $mg \cdot l^{-1}$	17.36	9.49	1.57	0.035
MCP, $g \cdot l^{-1}$	0.75	1.63	0.10	<0.001

[1] BD - basal diet; [2] BD + SDGCE - basal diet + SDGCE; [3] effect of SDGCE supplementation
TDHA - total dehydrogenase activeity; TVFA - total volatile fatty acids; NH_3-N - ammonia nitrogen; MCP - microbial crude protein

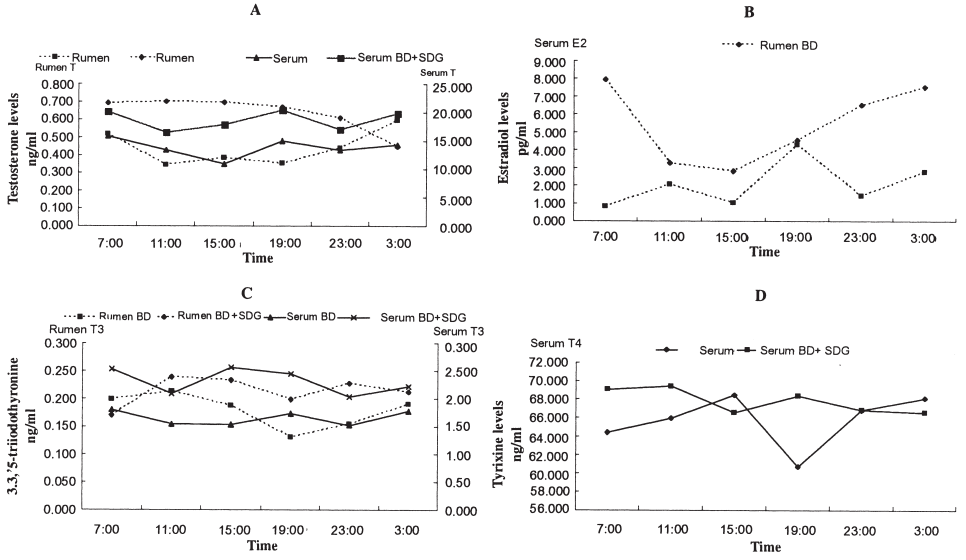

Figure 2. Effects of secoisolariciresinol diglucoside crude extract (SDGCE) on the concentrations of hormones in male goats. A - effects of SDGCE on the concentrations of testosterone (T) in serum and ruminal fluid. B - effect of SDGCE on the concentration of estradiol (E_2) in ruminal fluid. C - effects of SDGCE on the concentrations of 3, 3′, 5-triiodothyronine (T_3) in serum and ruminal fluid. D - effect of SDGCE on the concentration of thyroxine (T_4) in serum

The impacts of SDGCE on concentrations of hormones in male goats are presented in Figure 2. T concentrations were significantly increased at every time point throughout the sampling day in both serum ($P<0.01$) and ruminal fluid ($P<0.01$). The level of E_2 in serum was below the level of detection; however, its concentration in ruminal fluid was 5.431 ± 2.209 ng·l^{-1} on the BD and was remarkably decreased ($P<0.01$) by SDGCE. Positive effects of SDGCE on thyroid hormones were also observed. The T_3 levels were increased in both the rumen ($P<0.05$) and serum ($P<0.01$) by SDGCE; however, T_4 concentrations in serum were not significantly changed, and remained below the level of detection in ruminal fluid throughout this experiment.

DISCUSSION

Transformation and absorption of SDG in goats

The transformation and absorption of plant lignans have been studied in humans and rats, since ENL and END were first isolated from the urine of humans in 1980. It appears that the metabolic fate of lignans in non-ruminant animals may include several steps. Firstly, after deglycosylation, SDG is transformed to secoisolariciresinol (SCEO), and then demethylated and dehydroxylated in reactions catalysed by intestinal bacteria. SCEO is subsequently converted to END, which is further dehydroxylated to form ENL.

In our study, the significant increases in mammalian lignans were observed in both the rumen and blood circulation following SDGCE supplementation, suggested that ruminal microorganisms efficiently converted SDG to enterolignans, which then were absorbed. Moreover, the observation that after a 14-d exposure to SDGCE, the area under the curve for ENL in serum was nearly twice that of END, may indicate that ENL is the main circulatory lignan in goats. Bowey et al. (2003) and Knust et al. (2006) have demonstrated that when SDG was offered to human or human flora-associated rats, a larger amount of ENL was formed, whereas Tan et al. (2004) found that the urinary END concentration in rats was nearly 10 times that of ENL. Lignan profiles may therefore be dependent on the composition of the dominant intestinal microbiota in different animals.

Thus far, there is still some disagreement about whether SDG can be absorbed directly into the circulation. Our data demonstrates that SDG was present in serum; therefore, it might be absorbed directly through rumen wall. This is agreement with previous study in non-ruminant animals (Knust et al., 2006).

When the data of SDGCE treatment period for 21 and 28 d were compared with that for 14 d of sampling, the levels of both SDG and the 2 mammalian lignans in the rumen and circulation were similar; this may indicate that after a

14-d exposure to SDGCE, the transformation and absorption of the SDG in goats had reached equilibrium of a relatively stable state.

The NH_3-N concentration in ruminal fluid was significantly reduced by SDGCE, whereas the MCP level was remarkably increased. This contrasting response indicates that SDGCE may promote the utilization of non-protein nitrogen in the rumen. Simultaneously, the TVFA level and the (A+ B): P ratio in ruminal fluid were significantly increased by SDGCE, suggested that SDGCE may facilitate the anabolic metabolism of carbohydrates and change the fermentation pattern. These positive impacts on nitrogenous and anabolic metabolism were confirmed by an *in vitro* study carried out by Wang et al. (2007).

As the present study is the first to assess the effect of lignan supplementation on ruminants, there is currently a paucity of direct data relating to the physiological effects of SDG on rumen metabolism. Mao et al. (2007) demonstrated that daidzein, another important phytoestrogen, could dramatically change the acetate: propionate ratio (A:P), and that the amount of NH_3-N in the rumen was significantly reduced, whereas the MCP level tended to be higher. The causal mechanism appears to be the direct effect of daidzein on rumen microorganisms. As reviewed by Han (2006), in a study using water buffaloes fitted with permanent rumen and intestine fistulas, it was demonstrated that injection of daidzein (500 $mg \cdot d^{-1}$, 12 d) *via* duodenal cannulae increased serum T, rumen bacterial protein, ammonia nitrogen and total VFA. Thus, the effects of SDGCE on ruminal metabolism may be due, in part at least, to linseed lignans and/or their metabolites.

Previous studies have demonstrated that isoflavones, particularly daidzein and genistein, could influence the hypothalamus-pituitary-sexual gland axis in male animals (Liu et al., 1996) and the hypothalamus-pituitary- thyroid axis in male chickens (Han and Wang, 1993), and increases the serum T and thyroid hormones levels, respectively. Since the 3 types of lignans were found in serum in this study, the elevated T, T_3 and T_4 levels in the serum might be the outcome of a similar series of reactions. Previous research has demonstrated that the T and thyroid hormones in circulation could enter the rumen with saliva or *via* the rumen epithelium (Zhengkang, 2006). The increase in T and T_3 levels in the rumen observed in this study may have been caused by the elevated T and T_3 levels in serum. Even though serum T_4 levels were almost 30 times that of T_3, and low T_3 concentrations were found in the rumen, the concentrations of T_4 remained below the level of detection in ruminal fluid throughout this experiment; these observations are consistent with the findings of Hua and Han (1990). It has been reported that in filamentous fungi the transformation of T to E_2 may be catalysed by cytochrome P450 (Ahmed et al., 1996). This could explain the low level of E_2 found in the ruminal fluid of male goats. The reduced E_2 concentration and increased T level in ruminal fluid may have been caused by the inhibition of aromatase by END and ENL (Adlercreutz et al., 1993).

Sundby and Velle (1980) reported that rapid increases in T levels in Norwegian Red bulls aging 8 month old corresponded with rapid rates of weight gain. Meanwhile, the thyroid hormones T_3 and T_4 are associated with the regulation of metabolic rate and the modulation of other growth- and metabolism-related hormones (Brockman and Laarveld, 1986), and the increasing concentrations of triiodothyronine and thyroxine indicate a direct relationship with the nutritional level in ruminants that has been demonstrated by others researchers (Rhind et al., 2000; Delavaud et al., 2002). Thus, in our study, phytoestrogen compounds may have affected microbial activity and metabolism by increasing the levels of blood- and rumen-related hormones.

CONCLUSIONS

Male goats maintained on a basal diet were characterized by low levels of mammalian lignans and E_2 in ruminal fluid. Ruminal microorganisms efficiently converted secoisolariciresinol diglucoside (SDG) to enterodiol and enterolactone. In return, SDG and/or its metabolites may facilitate the utilization of non-protein nitrogen and carbohydrates in the rumen.

REFERENCES

Adlercreutz H., Bannwart C., Wähälä K., Mäkelä T., Brunow G., Hase T., Arosemena P.J., Kellis J.T., Vickery L.E., 1993. Inhibition of human aromatase by mammalian lignans and isoflavonid phytoestrogens. J. Steroid Biochem. Mol. Biol. 44, 147-153

Adlercreutz H., Van der J.W., Kinzel J., Avtalla H., Wähälä J., Mäkelä T., Hase T., Fotsis T., 1995. Lignan and isoflavonoid conjugates in human urine. J. Steroid Biochem. Mol. Biol. 52, 97-103

Ahmed F., Williams R.A.D., Smith K.E., 1996. Microbial transformations of steroids-X. Cytochromes P-450 11α-hydroxylase and C17-C20 lyase and A 1-Ene dehydrogenase transform steroids in *Nectria haematococca*. J. Steroid Biochem. Mol. Biol. 58, 337-349

Bowey E., Adlercreutz H., Rowland I., 2003. Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. Food Chem. Toxicol. 41, 631-636

Brockman R.P., Laarveld B., 1986. Hormonal regulation of metabolism in ruminants: a review. Livest. Prod. Sci. 14, 313-334

Chen J., Yang G.Y., Han Z.K., 1999. Effect of daidzein on serum testosterone and rumen digestion, metabolism in ruminants (in Chinese). Jiangsu Agr. Res. 20, 17-19

Clavel T., Henderson G., Alpert C.-A., Philippe C., Rigottier-Gois L., Doré J., Blaut M., 2005. Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. Appl. Environ. Microbiol. 71, 6077-6085

Delavaud C., Ferlay A., Faulconnier Y., Bocquier F., Kann G., Hilliard Y., 2002. Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. J. Anim. Sci. 80, 1317-1328

Dror Y., Mayevsky A., Bondi A., 1969. Some effects of starch on protein utilization by sheep. Brit. J. Nutr. 23, 727-735

Han Z.K., 2006. Neuroendocrine regulation of ruminal metabolism (in Chinese). J. Progr. Vet. Med. 27, 9-13

Han Z.K., Wang G.J., 1993. Studies of phytoestrogen (daidzein) affecting the growth and sex hormone levels in broilers. In: proceedings of World Conference on Animal Production: Edmonton (Canada) pp. 152-153

Han Z.K., Wang G., Yao W., Zhu W.Y., 2006. Isoflavonic phytoestrogens- new prebiotics for farm animals: a review on research in China. Curr. Issues Intest. Microbiol. 7, 53-60

Hua G., Han Z.K., 1990. Effects of thyroid powder tablet and methyl-thiouracil on the level of blood thyroid and metabolism in the rumen (in Chinese). J. Nanjing Agr. Univ. 13, 125-130

Knust U., Hull W.E., Spiegelhalder B., Bartsch H., Strowitzki T., Owen R.W., 2006. Analysis of enterolignan glucuronides in serum and urine by HPLC-ESI-MS. Food Chem. Toxicol. 44, 1038-1049

Liu G.T., Chen J., Chen W.H., 1996. Gender difference of feeding isoflavonic phytoestrogens on late pregnant sows on the growth in nursing piglets and endocrine function involved. Proceedings of the 8th AAAP Animal Science Congress, Japan, 2, 750-751

Mao S.Y., Zhu W.Y., Wang Q.J., Yao W., 2007. Effect of daidzein on *in vitro* fermentation by microorganisms from the goat rumen, Anim. Feed Sci. Tech. 136, 154-163

Morton M.S., Matos F.A., Abranches M.L., Correia R., Blacklock N., Chan P.S.F., Cheng C., Lloyd S., Wu C.P., Griffiths K., 1997. Measurement and metabolism of isoflavonoids and lignans in the human male. Cancer Lett. 114, 145-151

Rhind S.M., McMillen S.R., Duff E., Kyle C.E., Wright S., 2000. Effect of long-term feed restriction on seasonal endocrine changes in Soay sheep. Physiol. Behav. 71, 343-351

Sundby A., Velle W., 1980. Plasma concentration of testosterone in young bulls in relation to age rate of weight gain and stimulation with human chorionic gonadotrophin. J. Endocrinol. 86, 465-469

Tan K.P., Chen J.M., Ward W.E., Thompson L.U., 2004. Mammary gland morphogenesis is enhanced by exposure to flaxseed or its major lignan during suckling in rats. Exp. Biol. Med. 229, 147-157

Wang L.Q., 2002. Mammalian phytoestrogens: enterodiol and enterolactone. J. Chromatogr. B. 777, 289-309

Wang X.Z., Han Z.K., Zhou W., Wang G.J., 2007. Effects of linseed lignans on ruminal metabolism *in vitro*. In: Proceedings of the 6th Joint Symposium of China-Korea-Japan on Rumen Metabolism and Physiology, Hangzhou, (P.R. China) pp. 17

Weatherburn M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39, 971-974

Yang G.Y., Chen W.H., Chen J., Han Z.K., 1998. Detection of testosterone levels in rumen of male buffalo and research of its dynamic variations (in Chinese). J. Nanjing Agr. Univ. 21, 82-86

Zinn R.A., Owens F.N., 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66, 157-166