



A comparative study of the intestinal digestive characteristics of different feeds for Holstein cows

Shuai Han^{1†}, Fan Zhang^{2†}, Yanli Zhao³, Xiaoyu Guo³, Xiaowei Zhu¹ and Sumei Yan^{3*} 

Abstract

This experiment was conducted with lactating Chinese Holstein cows to study the nutritional value of local protein feed resources. A three-step method (TSP) and a modified three-step method (MTSP) were used to measure the *in vitro* digestibility of rumen undegraded protein (RUP) for 11 feedstuffs and correlation. Eleven experimental feeds were chosen and air-dried to investigate the effects of different growth periods and varieties on nutrition value and RUP digestibility. The small intestinal digestibility of RUP by TSP in concentrated feed was determined to be higher than that of roughage, approximately 65%. The highest concentrate (79%) was SBM (soybean meal), while the lowest was corn (65%). The proportions of DDGS (with soluble wine lees) and SFM (sunflower meal) were 70.9 and 74.9%, respectively. ASS (alfalfa mowed at the squaring stage) had the highest small intestinal digestibility of RUP (55%) among roughages, and WCS (whole-plant corn silage) had the lowest digestibility (40.5%). When the small intestinal digestibility of RUP was determined using the MTSP method, it exhibited similar results to the TSP method. Nevertheless, the values were generally higher, and there was a strong significant correlation between them ($R^2 = 0.967$, $P < 0.01$). The comparative study of these two methods help us have a better understanding of small intestine digestibility of different feeds, make a reasonable feed formula to effectively prevent diseases.

Keywords: Holstein cows, Intestinal digestibility, Rumen

Introduction

Dairy farmers often increase milk production by feeding cows a high-protein diet (concentrate), but high concentrations reduce the effective physical fiber content of the diet and decrease rumen fermentation, resulting in the accumulation of volatile fatty acids in the rumen and lowering rumen pH (Zhang et al. 2020). When rumen pH drops to a certain level it can cause subacute rumen acidosis (SARA) (Khalouei et al. 2020). Low rumen pH can cause the proliferation of Gram-negative bacteria in the

rumen, disintegrating and releasing large amounts of free lipopolysaccharide endotoxin (LPS), which can cause disease in cows (Guo et al. 2016). Therefore, it is necessary to investigate the different factors contributing to small intestinal digestibility of nondegraded protein in the rumen of dairy cows to gain a better understanding of the degradation of protein feeds in their digestive systems.

Many reports indicate that one of the most important nutritional limiting factors for ruminants is protein (Harmon and Swanson. 2020). Recent findings in the study of proteins in the small intestines of ruminants suggest that digestible crude protein (DCP). DCP in the small intestine is supplied primarily by combinations of microbial protein (MCP), rumen undegraded protein (RUP), and endogenous proteins (Corea et al. 2020). Among these, the RUP content has been observed to vary from 30% to

[†]Shuai Han and Fan Zhang contributed equally to this work.

*Correspondence: naruto1989@qq.com

³ College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China

Full list of author information is available at the end of the article



50%, MCP contents were stable, and endogenous proteins accounted for a small proportion of these three forms. Differences in RUP lead to compositional variation in DCP. The availability of nitrogen in growing ruminants is very important, and the rate and extent of protein degradation in the rumen directly determine this indicator. The small intestinal digestibility of RUP has an important effect on milk production and growth performance. RUP supplementation in ruminant diets improves the effective use of protein (Lobos et al. 2021). The supply of feed protein in the small intestine is obviously very important, and the digestibility of rumen undegraded protein in the small intestine is relevant to this supply (Kaur et al. 2011). Tests have shown that the small intestinal digestibility of feed of ruminants is related to many changing factors such as the feed type, processing technology, and origin (Lolli et al. 2021). Therefore, it is particularly important to study the effects of different raw materials in the rumen on their small intestinal digestibility.

Over the past several decades, much of the research on small intestinal digestibility has used mobile nylon bags and *in vitro* methods (De Boer et al. 1987; Promkot et al. 2007). This method of measurement entails placing feed into a nylon bag in the rumen of the ruminant through a fistula and incubating it in the rumen to determine the degree of rumen degradation of the feed proteins (Ari-sya et al. 2019). A three-step *in vitro* procedure measures feed N, which escapes rumen degradation and digestion (Calsamiglia and Stern 1995). However, cows should be equipped with ruminal and duodenal cannulas to determine the RUP of the mobile nylon bag technique. The physiologies of experimental and normal mice are undeniably different. Calsamiglia and Stern expressed the view that the three-step *in vitro* procedure (TSP), using a comprehensive rumen nylon bag technique and *in vitro* methods, simulates the physiological conditions of ruminants, is easy to standardize, and is economical (Calsamiglia and Stern 1995). Gargallo et al. developed a modified three-step *in vitro* procedure (MTSP) to improve the TSP and reported that the two methods are highly relevant ($R^2 = 0.98$, $P < 0.001$). MTSP requires less labor and time, reduces pollution, and promotes commercial trials (Gargallo et al. 2006). The aim of the present study is to contribute to the understanding of the small intestinal digestibility RUP of different protein sources using TSP and MTSP and to provide a theoretical basis for a simplified method to examine small intestinal digestibility.

Results

Nutrient composition

The chemical compositions of the feeds used in this study are presented in Table 1. All 11 dry matter (DM) feeds

Table 1 The contents of DM, CP, NDF, ADF and ADL in raw feed (%)

Feed stuff	DM	CP	NDF	ADF	ADL
Corn	85.54	8.69	15.57	1.78	0.13
SBM	87.49	43.15	15.34	6.37	0.93
DDGS	95.70	24.49	46.18	16.86	8.63
SFM	96.82	29.10	45.96	23.68	9.71
CS	95.20	3.84	75.01	42.12	4.38
WCS	94.27	4.69	69.63	41.37	4.34
CH	95.43	12.54	63.53	35.72	6.14
CW	94.85	8.62	66.58	38.55	5.24
ASS	95.14	10.58	65.06	37.14	5.69
AFS	95.00	9.60	65.82	37.84	5.47
APS	95.00	9.60	65.82	37.84	5.47

SBM soybean meal, DDGS distillers dried grains with soluble, SFM sunflower meal, CS corn stalk, WCS whole-plant corn silage, CH Chinese wildrye, CW Chinese wildrye, ASS alfalfa mowed at squaring stage, AFS full-bloom stage, APS pod stage, DM dry matter, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin

were similar except for corn and soybean meal (SBM). Neutral detergent fiber (NDF,75%) was the highest in corn stalk (CS), and so was acid detergent fiber (ADF, 42.1%). The lowest ADF (1.8%) was in corn, and SBM had the lowest NDF (15.3%). The ranking of crude protein (CP) from low to high was CS, corn haylage (CH), whole-plant corn silage (WCS), Chinese wildrye (CW), corn, AH, distillers dried grains with solubles (DDGS), sunflower meal (SFM), and SBM. Three varieties of alfalfa mowed at the squaring stage, full-bloom stage, and pod stage were measured. Obviously, the CP content decreased as the growth period progressed, while the PDF, ADF, and acid detergent lignin (ADL) contents increased.

Determination of CP in the rumen

Figure 1 shows the 16 h degradation rate of CP in the rumen for different feed ingredients. The results show that CP in concentrate feeds (Corn, SBM, DDGS, SFM) degraded faster in the rumen compared to roughage, with SBM reaching the fastest degradation rate of 68.29%. However, the degradation rate of ASS in roughage was higher than that in other types of feed, reaching 49.19%.

The small intestinal digestibility of RUP

Original TSP and modified TSP-related data measurements are shown in Table 2. The small intestinal digestibility of RUP is presented in Table 3 and Fig. 2. In the TSP method, SBM and SFM were more highly digested than others. The small intestine digestibility of RUP in

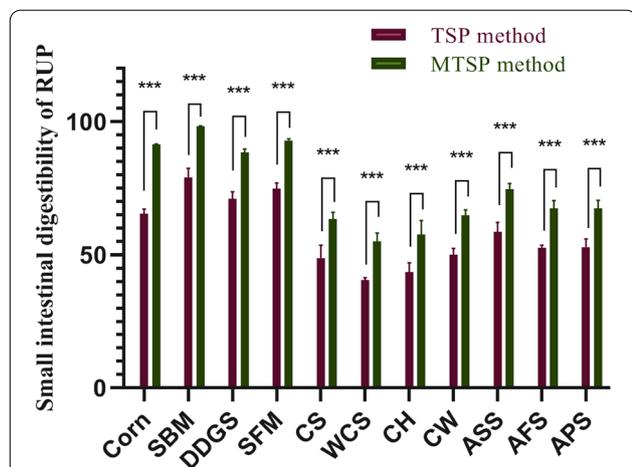


Fig. 1 Sixteen-hour degradation rate of feed ingredients CP in the rumen. SBM, soybean meal; DDGS, distillers dried grains with soluble; SFM, sunflower meal; CS, corn stalk; WCS, whole-plant corn silage; CH, Chinese wildrye; CW, Chinese wildrye; ASS, alfalfa mowed at squaring stage; AFS, full-bloom stage; APS, pod stage

Table 2 RUP small intestine digestibility-related data

Feedstuff	Original TSP		Modified TSP	
	TCA soluble N in (%)	TCA soluble N out (%)	Amount of RUP in (g)	Amount of RUP put (g)
Corn	1.24	1.90	0.0647	0.0055
SBM	9.58	12.13	0.2609	0.0022
DDGS	3.90	5.49	0.1689	0.0194
SFM	3.09	4.13	0.1248	0.0088
CS	0.40	0.81	0.0081	0.0030
WCS	0.31	0.74	0.0095	0.0045
CH	0.26	0.63	0.0063	0.0028
CW	0.41	0.87	0.0135	0.0050
ASS	0.76	1.39	0.0171	0.0050
AFS	0.65	1.26	0.0137	0.0049

WCS whole-plant corn silage, CH Chinese wildrye, CW Chinese wildrye, ASS alfalfa mowed at squaring stage, AFS full-bloom stage, APS pod stage

concentrated feed is approximately 65% and is higher than that of roughage. ASS has the highest RUP intestinal digestibility in roughage, approximately 55%, while WCS has the lowest intestinal digestibility of all, approximately 40.5%. This finding also applies to the MTSP method.

Additionally, the 11 kinds of feeds were analyzed using paired t-tests comparing the different methods. All experimental values of MTSP are higher than those of the TSP method, and the difference was significant according to the paired t-tests.

Table 3 The small intestinal digestibility of RUP

Feedstuff	TSP method	MTSP method
Corn	65.38 ± 1.78c	91.41 ± 0.21b
SBM	79.01 ± 3.40a	98.22 ± 0.24a
DDGS	70.94 ± 2.68bc	88.49 ± 1.26b
SFM	74.85 ± 2.13ab	92.98 ± 0.56ab
CS	48.89 ± 4.63ef	63.47 ± 2.48de
WCS	40.55 ± 0.90g	55.01 ± 3.17f
CH	43.57 ± 3.44gf	57.50 ± 5.32ef
CW	49.92 ± 2.46ef	64.80 ± 2.04d
ASS	58.51 ± 3.64d	74.50 ± 2.26c
AFS	52.68 ± 0.88de	67.45 ± 2.93d
APS	52.84 ± 3.08de	67.35 ± 3.10d
Between different feeds	$P < 0.001$	
SEM	57.92A	74.65B
TTEST	$P < 0.001$	

SBM soybean meal, DDGS distillers dried grains with soluble, SFM sunflower meal, CS corn stalk, WCS whole-plant corn silage, CH Chinese wildrye, CW Chinese wildrye, ASS alfalfa mowed at squaring stage, AFS full-bloom stage, APS pod stage, TTEST paired t-test

A–B Means within a row with different superscripts differ significantly ($P < 0.01$).

a – g Means within a column with different superscripts differ significantly ($P < 0.001$).

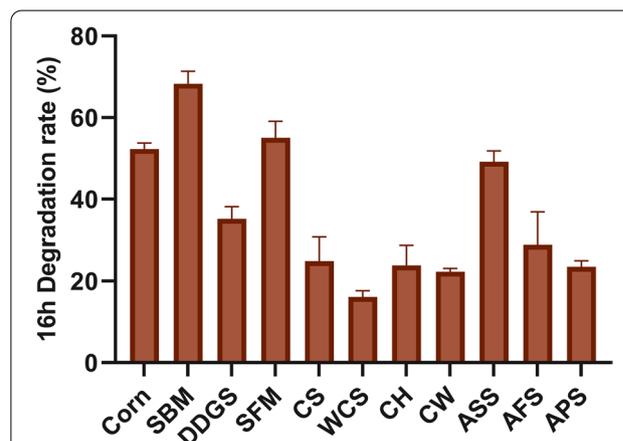


Fig. 2 The intestinal digestibility of RUP. SBM, soybean meal; DDGS, distillers dried grains with soluble; SFM, sunflower meal; CS, corn stalk; WCS, whole-plant corn silage; CH, Chinese wildrye; CW, Chinese wildrye; ASS, alfalfa mowed at squaring stage; AFS, full-bloom stage; APS, pod stage

Correlation analysis

The data analyses for the small intestinal digestibility of RUP showed that a high positive correlation exists between the TSP method and the MTSP method when considering the small intestinal digestibility of RUP ($Y = 1.1864X + 5.7186, R^2 = 0.967, P < 0.0001$). There

Table 4 Regression analysis between the content of CP in feedstuffs after 16 h of incubation in the rumen and after intestinal digestion

Type of feed	Regression equation	R ²	n	p
Concentrate feed	$Y = -0.0147X^2 + 0.2369X + 0.1477$	$R^2 = 0.3350$	4	$P = 0.1594$
Coarse feed	$Y = -0.1560X^2 + 0.3754X + 0.2344$	$R^2 = 0.0551$	15	$P = 0.3506$
Concentrate and coarse feed	$Y = -0.0112X^2 + 0.1853X + 0.2625$	$R^2 = 0.5787$	19	$p < 0.0001$

Y, protein content of the rumen degradation residue of the feed material (%); X, protein content of the residue after small intestine digestion with the MTSP method (%)

was a good correlation between the TSP methods and MTSP methods for the different feedstuffs tested. However, there was no significant correlation between CP content of feedstuffs after incubation for 16 h in the rumen and after intestinal digestion (Table 4).

Discussion

In this study, CP content of SBM (43.15) in raw feeds was similar to that reported by previous study (Yao et al. 2007; Promkot et al. 2007). CP content of corn was comparable to that reported by C. Mikolayunas (Antoniewicz et al. 1992), while that of DDGS was within the range reported by D.H. Kleinschmit (Kleinschmit et al. 2007). A.P. Santos reported that the high RUP sources compared with SBM were distillers' dried grains (DDG), DDGS, and heated SBM (HSBM) (Santos et al. 1998). The CP degradability *in vitro* pepsin-pancreatin digestibility of feedstuffs for ruminants has been reported extensively in recent years. They reached the same conclusion that the small intestinal digestibility of RUP in concentrated feed is higher than for roughages. Kleinschmit used TSP method to study the small-intestinal digestibility of DDGS from different producing areas after 12 h of incubation in the rumen and concluded that 70.9% resulted from the TSP method in this test and slightly higher with the MTSP method (Kleinschmit et al. 2007). This may be caused by different incubation times in the rumen. C. Promkot reported that the small intestinal digestibility values of cassava hay, SBM, and DDG were 70.4%, 79.8%, and 71.7%, respectively (Promkot et al. 2007). The Nutrient Requirements of Dairy Cattle investigated the small intestinal digestibility of RUP of DDGS, corn, SBM, CS, and alfalfa meal, with values of 80, 90, 93, 70, and 75%, respectively (NRC 2001). Under this test condition, the TSP method result was slightly lower compared to NRC, while the MTSP method was identical to NRC. High-protein, low-fiber feed is easily digested and utilized by the small intestine (Zhao and Yuanjun 2017). The ruminal degradation rate of corn stalk protein is lower than that of corn stalk silage, which is consistent with this experiment.

The small intestinal digestibility of RUP measured by TSP in roughages was lower than in concentrated feed. This may be due to the forage protein being degraded in the rumen and the remainder combined with lignin, which is difficult to digest. The MTSP method provided similar results, but the measured value was slightly higher than for the TSP method. A possible explanation is that polypeptides and small peptides in the residues were precipitated by trichloroacetic acid after incubation in the rumen. In this experiment, data analyses for the small intestinal digestibility of RUP exhibited a high positive correlation between the TSP method and the MTSP method for the small intestinal digestibility of RUP, and the results of the correlation test and the paired t-test were consistent. Similar results were reported by Gargallo reported, $MTSP = 1.37 \times TSP - 15.45$ ($R^2 = 0.98$, $P < 0.001$) (Gargallo et al. 2006).

Wang reported that the RUP in 13 concentrates was measured using TSP and MTSP (Wang and Fang 2012). Their results are lower than the results of this test, which may be related to factors such as feed type and origin. It was reported that the improvement in the three-step *in vitro* measurement results was 0.8383 compared with the measurement results from the mobile nylon bag method.

Kleinschmit used the TSP method to study the small-intestinal digestibility of DDGS from different producing areas after 12 h of incubation in the rumen and concluded that the digestibility was 70.94% using the TSP method and slightly higher using the MTSP method (Kleinschmit et al. 2007). This may have been caused by different incubation times in the rumen.

The small intestinal digestibility of RUP in concentrated feed was higher than that in roughages. SBM had the highest (79.0%). The small intestinal digestibility of RUP and WCS was the lowest (40.5%) using TSP. The small intestinal digestibility of RUP determined using MTSP was slightly higher than for TSP, and there was a strong significant correlation between them. Compared with TSP, the modifications could measure a maximum of 120 samples at one time, faster and more efficient than using TSP. MTSP also avoids the use of trichloroacetic acid, which is corrosive, toxic, and pollutes the

environment, facilitating the assessment of feed digestibility RUP.

Conclusion

In summary, rumen and intestinal digestion of feed in dairy cows are a complex process, and studies on the small intestinal digestibility of RUP in dairy cows can help to understand the differences in their digestion of different feeds. Providing cows with a more complete and balanced diet can improve disease resistance and reduce the incidence of disease. Such studies also contribute to a theoretical basis for the dietary preparation of lactating cows.

Materials and methods

Experimental animals and design

Four Chinese Holstein cows with an average BW of 550 (± 28) kg, 20 (± 2.3) kg daily milk production, and 156 (± 8.2) lactation days at the start of the experiment were used for the study. Total mixed ration (TMR) and forage were used in a concentration ratio of 45:55 (Table 5). The cows were fed ad libitum twice daily at 06:30 am and 04:30 pm. Freshwater was ensured during the experimental period. The feed was started ten days before the experiment. In this experiment, eleven types of concentrates that can pass through a 2.5 mm sieve were prepared. All feeds were from Hohhot Tu mo te Zuo Qi. The eleven types of concentrates were corn, SBM (soybean meal), DDGS (distillers dried grains with solubles), SFM (sunflower meal), CS (corn stalk), WCS (whole-plant corn silage), CH (corn haylage), CW (Chinese wildrye), ASS (alfalfa mowed at squaring stage), AFS (full-bloom stage), and APS (pod stage). Three varieties of ASS, AFS, and APS were chosen to investigate the effect of different growth periods and varieties on nutritional value and RUP digestibility. Testing was carried out at the Hohhot To mo te Zuo Qi Xiao dan dam Ranch.

Chemical analysis

The supplied sample was dried at 65 °C for 5 h to remove its initial moisture. The samples were analyzed for DM according to the moisture measurement method (Seymour et al. 2019). This experiment used the Kjeldahl method (AOAC, 1990) to determine CP and used the methods of Van Soest to determine PDF and ADF and ADL (Soest and Jung 1995).

Original TSP

There were three parallels per cow. Nylon bags were incubated one hour before morning feeding and were removed, washed, and dried at 65 °C to constant weight. The CP level of the residue was measured after the feedstuff was preincubated in the rumen for 16 h and rinsed with tap water.

Table 5 Ingredients and nutritive value of experimental diets

Ingredients	%	Nutritive value	%
Corn	25.36	DM	87.20
Soybean meal	6.56	NEL*(MJ/kg)	5.92
Sunflower cake	3.26	CP	10.65
Cottonseed meal	5.40	Ca	0.62
Bran	1.69	P	0.37
Sodium bicarbonate	0.52	NDF	45.35
Calcium phosphate dibasic	0.85	ADF	27.83
Salt	0.52	ADL	6.21
Powder	0.39	AIA	1.37
Premix	0.45		
Corn silage	55		
Total	100		

DM Dry matter, NEL Net Energy for Lactation, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, AIA acid insoluble

The residue (15 mg N) was introduced into a 50 mL centrifuge tube with 10 mL of HCl (pH 1.9) containing 1 g/L pepsin (Sigma P-7012). The tube was incubated for 1 hour at 38 °C with constant rotation before 13.5 mL trypsin solution (pH 7.8 phosphate buffers, 50 ppm thymol, 3 g/L trypsin (Sigma P-7545)) was added and incubated for 24 h with constant rotation at 38 °C. After 24 h, 3 mL 100% trichloroacetic acid was added and left for 15 min before the supernatant was collected by centrifugation (10000 rpm, 15 min). The Kjeldahl nitrogen method (AOAC, 1980) was used to analyze the N content of the supernatant. The calculation formula is as follows:

$$\% \text{ digestibility} = (\% \text{ TCA soluble N in, g} / \% \text{ TCA soluble N out, g}) \times 100\%.$$

Modified TSP

The samples test were also subjected to the modified TSP. Briefly, 1~2 g of the residue was weighed into three duplicate bags for each sample after incubation in the rumen for 16 h. The residue was sealed in a nylon bag (R510), which was placed in a Daisy II flask, with each flask allowed to hold 30 nylon bags. The solution in the culture flask was 2 L of a hydrochloric acid solution with a pH of 1.9 containing 1 g/L pepsin (P-7000, Sigma). The samples were incubated at 39 °C for 1 h. After that, the nylon bag was removed and rinsed and 2 L of a trypsin solution (0.5 mol/L phosphate buffer, 50 ppm thymol, 3 g/L trypsin (P-7545, Sigma)) was added and cultured at 39 °C for 24 h. After 24 h, the nylon bag was removed and rinsed, and held constant at 65 °C. The CP content in the residue was determined.

The formula for the digestibility of RUP is as follows:

$$\% \text{ digestibility} = \left[\frac{(\text{amount of RUP in, g} - \text{amount of RUP put, g})}{\text{amount of RUP in, g}} \right] \times 100\%.$$

Statistical analysis

The data were processed using the GLM program in SAS 9.0. Data analysis used a completely randomized design paired t-test. The TSP method and MTSP method were treated as random effects in the experiment. Statistical evaluation of eleven feedstuffs was calculated with a variance test. The CORR procedure in SAS was utilized to examine the relationship between standardized TSP and MTSP methods in determining the small intestinal digestibility of RUP.

Abbreviations

ADF: Acid detergent fiber; ADL: Acid detergent lignin; AFS: Full-bloom stage; APS: Pod stage; ASS: Alfalfa mowed at squaring stage; CF: Crude fiber; CH: Chinese wildrye; CP: Crude protein; CS: Corn stalk; CW: Chinese wildrye; DM: Dry matter; DDGS: Distillers dried grains with soluble; MCP: Ruminant microbial proteins; MTSP: Modified three-step method; NRC: Nutrient Requirements of Dairy Cattle; NDF: Neutral detergent fiber; RUP: Ruminant undegradable proteins; SBM: Soybean meal; SFM: Sunflower meal; TMR: Total mixed ration; TSP: Three-step method; WCS: Whole-plant corn silage.

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Not applicable.

Authors' contributions

Shuai Han: AB/FG. Fan Zhang: AB/FG. Sumei Yan: FG. Xiaowei Zhu: ES. Yanli Zhao: ES. Xiaoyu Guo: ES. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study and its experimental procedures were approved by the institutional animal care and use committee of Inner Mongolia Medical University (approval no. SYDWZX202103001). All animal housing and experiments were conducted in strict accordance with the institutional guidelines for the care and use of laboratory animals.

Consent for publication

Not applicable.

Competing interests

I hereby declare that the coauthors of this manuscript, familiar with its content, have given their consent to publish the manuscript in the presented form in *Animal Diseases*, and all authors declare that there is no conflict of interest.

Author details

¹Laboratory Animal Center of Inner Mongolia Medical University, Inner Mongolia Medical University, Hohhot, China. ²Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin, China. ³College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China.

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