Effects of Replacing Alfalfa with Rice Straw or Corn Stover on Nitrogen Metabolism, Serum Physiological and Biochemical Parameters, and Liver and Kidney Histomorphology in Dairy Cows: A Postprint

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Abstract

This experiment was conducted to investigate the effects of replacing alfalfa with rice straw or corn stover on nitrogen metabolism, serum physiological and biochemical indices, and histomorphological changes in the liver and kidney of dairy cows. Forty-five healthy multiparous Holstein dairy cows in mid-lactation were selected and randomly divided into 3 groups with 15 cows per group. Each group was fed diets containing different roughages with a concentrate-to-forage ratio of 55:45: alfalfa group (23% alfalfa hay + 7% Chinese wildrye, AH group), corn stover group (30% corn stover, CS group), and rice straw group (30% rice straw, RS group). The experimental period lasted for 12 weeks. The results showed that fecal nitrogen excretion in CS and RS groups was significantly higher than that in AH group ($P<0.05$), and urinary nitrogen excretion in RS group was significantly higher than that in AH and CS groups ($P<0.05$). Serum urea nitrogen and creatinine contents in RS group were significantly higher than those in AH group ($P<0.05$). Serum total cholesterol content in RS group was significantly higher than that in AH and CS groups ($P<0.05$). Serum alanine aminotransferase activity in CS group was significantly higher than that in AH group ($P<0.05$), while serum aspartate aminotransferase activity was significantly higher than that in AH and RS groups ($P<0.05$). Liver and kidney tissue sections from all groups showed clear structure with no obvious abnormalities. It can be concluded that the increased fecal and urinary nitrogen excretion in dairy cows fed corn stover or rice straw reduced nitrogen utilization efficiency. Although there were no significant changes in liver and kidney histomorphology among groups, feeding diets containing corn stover or rice straw could elevate serum indices reflecting liver and kidney health function in dairy cows.

Full Text

Effects of Alfalfa Replacement by Rice Straw or Corn Stover on Nitrogen Metabolism, Serum Physiological-Biochemical Indices, and Histomorphology of Liver and Kidney in Dairy Cows

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Abstract

This study investigated the effects of replacing alfalfa with rice straw or corn stover on nitrogen metabolism, serum physiological-biochemical indices, and histomorphological changes in the liver and kidney of dairy cows. Forty-five multiparous, healthy Holstein dairy cows in mid-lactation were randomly allocated to three groups (n=15). Each group received diets with different roughage sources but identical forage-to-concentrate ratios of 55:45: alfalfa group (23% alfalfa hay + 7% Chinese wild rye hay, AH group), corn stover group (30% corn stover, CS group), and rice straw group (30% rice straw, RS group). The 12-week trial revealed that fecal nitrogen excretion was significantly higher in CS and RS groups compared to AH group ($P<0.05$), while urinary nitrogen excretion was significantly higher in RS group than in both AH and CS groups ($P<0.05$). Serum urea nitrogen and creatinine concentrations were significantly elevated in RS group versus AH group ($P<0.05$), and serum total cholesterol was significantly higher in RS group compared to AH and CS groups ($P<0.05$). Alanine aminotransferase activity was significantly higher in CS group than AH group ($P<0.05$), whereas aspartate aminotransferase activity was significantly higher in CS group than both AH and RS groups ($P<0.05$). Histological examination revealed clear tissue structure in liver and kidney sections across all groups with no obvious abnormalities. These findings indicate that feeding corn stover or rice straw increased fecal and urinary nitrogen excretion, thereby reducing nitrogen utilization efficiency. Although no significant morphological changes were observed in liver and kidney tissues, diets containing corn stover or rice straw elevated serum markers reflecting liver and kidney functional status.

Keywords: dairy cows; nitrogen metabolism; liver; serum

Over the past decade, China's dairy industry has experienced rapid development, with substantial improvements in per-cow milk production largely attributed to the application of high-quality alfalfa as a forage source. However, domestic alfalfa resources are limited, forcing heavy reliance on imports that escalated

dramatically from 2008 to 2015, reaching nearly 1.21 million tons annually by 2015. This dependency has become a constraining factor for sustainable development of China' s dairy industry. Concurrently, China possesses abundant crop straw resources, with an annual output of approximately 800 million tons, including about 200 million tons each of corn stover and rice straw, which represent the main types of crop residues. Consequently, effective utilization of these straws as dairy cow roughage has attracted increasing attention. Nevertheless, the application of crop straws in dairy feeding is limited by their inherently low nutritional value, particularly in metabolizable energy, crude protein (CP), trace elements, and vitamins. Previous research demonstrated that replacing alfalfa with rice straw or corn stover significantly reduced milk yield and milk protein synthesis, primarily due to insufficient nutrients, especially energy deficiency, which suppressed metabolic processes. Studies on amino acid metabolism revealed that rice straw-based diets decreased amino acid flow in the digestive tract, reducing free amino acid uptake by the mammary gland and consequently increasing milk urea nitrogen content. Energy deficiency from straw diets also disrupted glucose metabolism, constraining lactose synthesis. However, systematic research on the effects of straw feeding on nitrogen metabolism and animal health remains scarce. Therefore, this experiment investigated how corn stover or rice straw replacement of alfalfa affects nitrogen metabolism to elucidate mechanisms underlying reduced milk protein synthesis, and examined serum physiological-biochemical indices plus liver and kidney histomorphology to assess impacts on nutrient metabolism and health status.

1.1 Experimental Animals, Diets, and Design

Forty-five multiparous Holstein dairy cows with average body weight of $(607\$\pm56)kg, daysinmilkof(164\pm25)d, andmilkyieldof(29.7\pm\$4.7)$ kg/d were randomly assigned to three groups (n=15) based on milk production and lactation stage. All diets maintained a 55:45 forage-to-concentrate ratio (dry matter basis), were isonitrogenous but not isoenergetic, and contained identical concentrate and corn silage components, differing only in roughage source: alfalfa hay group (23% alfalfa hay + 7% Chinese wild rye hay, AH group), corn stover group (30% corn stover, CS group), and rice straw group (30% rice straw, RS group). Diets were fed as total mixed rations (TMR) prepared using a TMR mixer (9SJW-300). Diet composition and nutrient levels are presented in Table 1 .

Following a 2-week adaptation period, the 12-week experimental trial commenced. Cows were housed in tie-stall barns with good ventilation, fed and milked three times daily at 06:30, 14:00, and 20:00. Pipeline milking systems were used, and water was freely available. The experimental protocol was approved by the Animal Care Committee of Zhejiang University (Hangzhou, China), and all procedures followed institutional regulations.

1.2 Sampling and Analysis

During the 12-week sampling period, feed and milk samples were collected continuously for 3 days in weeks 6 and 10, with concurrent recording of feed intake and milk yield. Milk samples were collected at a 4:3:3 ratio from morning, afternoon, and evening milkings to obtain approximately 50 mL daily. Samples were preserved with potassium dichromate at 0.06% (w/v) and stored at 4°C until analysis of milk protein and nitrogen content by infrared spectroscopy.

Fecal (300 g) and urine (15 mL) samples were collected before feeding in the morning, afternoon, and evening during one day in weeks 6 and 10. All feed and fecal samples were dried at 65°C, ground sequentially through 2-mm and 1-mm screens, and stored at 4°C. Urine samples were mixed with 0.036 mol/L H_2SO_4 at a 1:4 ratio and stored at -20°C. Dietary samples were analyzed for dry matter (105°C for 5 h), crude ash (carbonized then ashed in muffle furnace at 550°C for 2 h), crude protein (Kjeldahl method using FOSS Kjeltec 8400, Denmark), acid detergent fiber (ADF), and neutral detergent fiber (NDF) using the Van Soest fiber method with an ANKOM A2000i fiber analyzer (USA) according to AOAC (1990) methods. Total nitrogen in feces and urine was determined by the Kjeldahl method as described in AOAC (1990).

Indigestible NDF was used as an internal marker to estimate daily fecal output. Approximately 3 g of diet or fecal sample (ground through 1-mm screen) was weighed into 25- m dialysis bags and incubated in the rumen for 12 days, with residual content measured to calculate indigestible NDF. Daily fecal output was estimated as: fecal output = indigestible NDF intake / indigestible NDF concentration in feces.

Urinary volume was estimated using creatinine concentration: urinary volume (L/d) = body weight × 29 / creatinine concentration (mg/L).

Blood samples (10 mL) were collected from the jugular vein 3 h after morning feeding on day 5 of weeks 3, 6, 9, and 12 using vacuum coagulation-promoting tubes. Serum was prepared by centrifugation at 3000×g for 15 min and analyzed for physiological-biochemical indices using an automatic biochemical analyzer with kits from Nanjing Jiancheng Bioengineering Institute. Measured parameters included glucose (colorimetric method), total protein (TP, colorimetric method), urea nitrogen (urease method), triglycerides (TG, colorimetric method), non-esterified fatty acids (NEFA, colorimetric method), β-hydroxybutyric acid (BHBA, colorimetric method), albumin (ALB, colorimetric method), globulin (GLB, colorimetric method), total cholesterol (TCH, triglyceride oxidase method), alkaline phosphatase (ALP, colorimetric method), alanine aminotransferase (ALT, colorimetric method), aspartate aminotransferase (AST, colorimetric method), total bilirubin (T-BIL, colorimetric method), and creatinine (colorimetric method).

Upon completion of the feeding trial, six cows per group were slaughtered at the Hangzhou Fuyang Animal Disposal Facility. Animals were euthanized following

standard procedures, and liver and kidney tissues were immediately excised. Parenchymal organs were trimmed to 2 cm × 2 cm × 1 cm blocks, while hollow organs were cut into 2 cm × 1 cm pieces and fixed in 4% formaldehyde for 18 h. After trimming, tissues were refixed for 18-24 h, then rinsed under running water for 6-12 h. Dehydration was performed in ascending ethanol series (70%, 80%, 85%, 90%, 95%, 100% (I), and 100% (II)) for 2 h each, followed by clearing in xylene for 0.5 h (repeated once), and paraffin embedding (paraffin I and II for 2 h each) using an automated processor. Embedded blocks were sectioned at 8 m, dried at 60°C for 8-12 h, and stained with hematoxylin-eosin (HE) before mounting with neutral balsam.

1.3 Statistical Analysis

Nitrogen metabolism and serum physiological-biochemical indices were analyzed using the PROC MIXED procedure in SAS V8.1 according to a completely randomized block design with repeated measures. Time, diet, and their interaction were included as fixed effects, with cow as a random effect. The minimum Akaike information criterion was used for covariance structure selection in repeated measures analysis. Results are presented as least squares means. Histological sections were photographed at medium magnification (5×), with one representative slide selected per sample. Significance was declared at P$ $0.05, and trends were noted at 0.05<P<0.10.

Results

2.1 Effects on Nitrogen Metabolism

Nitrogen metabolism results are summarized in Table 2 . Nitrogen intake was significantly higher in AH group compared to RS group (P<0.05), with no significant difference between CS group and the other two groups (P>0.05). Fecal nitrogen excretion and its proportion of nitrogen intake were significantly greater in CS and RS groups than in AH group (P<0.05). Urinary nitrogen excretion and its proportion of nitrogen intake were significantly higher in RS group compared to AH and CS groups (P<0.05), while no significant difference existed between AH and CS groups (P>0.05). Milk nitrogen content was significantly higher in AH group than in CS and RS groups (P<0.05). Retained nitrogen was significantly greater in AH and CS groups versus RS group (P<0.05). Significant time effects were observed for nitrogen intake, fecal nitrogen excretion, urinary nitrogen excretion, and milk nitrogen content (P<0.05). Significant time × diet interactions were detected for fecal nitrogen excretion and its proportion of nitrogen intake (P<0.05).

2.2 Effects on Serum Physiological-Biochemical Indices

Serum physiological-biochemical indices are presented in Table 3 . No significant differences were observed among groups for serum glucose concentration

(P>0.05). Although AH group showed the highest serum total protein content, differences among groups were not significant (P>0.05). Serum urea nitrogen concentration was significantly higher in RS group compared to AH group (P<0.05). Serum triglyceride concentration was significantly elevated in CS group versus RS group (P<0.05), while no significant differences were detected for serum non-esterified fatty acids or β-hydroxybutyric acid among groups (P>0.05). Serum total cholesterol concentration was significantly higher in RS group than in AH and CS groups (P<0.05). Alanine aminotransferase activity was significantly greater in CS group compared to AH group (P<0.05), whereas aspartate aminotransferase activity was significantly higher in CS group than in both AH and RS groups (P<0.05). Total bilirubin concentration was significantly elevated in CS group versus RS group (P<0.05), and serum creatinine concentration was significantly higher in RS group compared to AH group (P<0.05). All measured parameters except albumin/globulin ratio showed significant time effects (P<0.05). Significant time × diet interactions were observed for serum urea nitrogen, triglycerides, non-esterified fatty acids, β-hydroxybutyric acid, and total cholesterol concentrations (P<0.05).

2.3 Effects on Liver and Kidney Histomorphology

Liver histomorphology is illustrated in Figure 1 [Figure 1: see original paper]. All three groups exhibited clear hepatic architecture without obvious abnormalities. Hepatic lobule boundaries were distinct, hepatocyte plate structure was normal, hepatic sinusoids were clearly demarcated, and Kupffer cell numbers appeared normal. Portal triad structures including interlobular arteries, veins, and bile ducts were essentially normal, and hepatocyte morphology was intact.

Kidney histomorphology is shown in Figure 2 [Figure 2: see original paper]. All groups displayed normal renal architecture with intact cortical glomeruli, proximal and distal convoluted tubules. Medullary collecting tubules were clearly bounded, and interstitial connective tissue quantity was normal.

Discussion

3.1 Impacts on Nitrogen Metabolism

Previous studies demonstrated that cows fed straw-based diets exhibited reduced milk yield and milk protein production, with rice straw diets significantly decreasing milk protein content and consequently reducing nitrogen efficiency for milk synthesis. This experiment further investigated nitrogen metabolism patterns, revealing that cows fed corn stover and rice straw diets had significantly greater fecal nitrogen excretion, while rice straw-fed cows showed significantly increased urinary nitrogen excretion compared to alfalfa-fed cows. Excessive nitrogen waste emissions not only pose environmental concerns but may also adversely affect cow health. Research indicates that elevated fecal nitrogen excretion in straw-fed groups correlates with apparent crude protein digestibility, suggesting that increased fecal nitrogen loss primarily results from poor

nitrogen digestibility. The heightened urinary nitrogen excretion in rice straw group aligned with serum urea nitrogen concentrations. Previous reports found that rice straw diets significantly increased rumen ammonia nitrogen and milk urea nitrogen concentrations. Additionally, urinary nitrogen excretion correlates with milk urea nitrogen content, and both rumen ammonia nitrogen and serum urea nitrogen reflect nitrogen utilization efficiency and influence milk protein synthesis. Therefore, increased urinary nitrogen excretion in rice straw-fed cows likely stems from inefficient microbial utilization of rumen ammonia nitrogen, leading to excess nitrogen entering the urea cycle, elevating serum urea nitrogen, and subsequent renal excretion. Although nitrogen efficiency for lactation did not differ between RS and CS groups, the significantly higher urinary nitrogen excretion in RS group resulted in lower nitrogen retention compared to CS group.

3.2 Impacts on Serum Physiological-Biochemical Indices

Earlier research showed that rice straw diets significantly reduced amino acid and glucose concentrations in tail artery and mammary vein blood, yet this experiment found no significant differences in jugular vein serum glucose. This discrepancy likely arises from differences in blood vessel type, sampling location, and collection timing. Furthermore, serum glucose may not sensitively reflect energy metabolism due to homeostatic regulation. Serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphase activities, and total cholesterol and bilirubin concentrations serve as important indicators of hepatic metabolic dysfunction. Serum total cholesterol also reflects lipid metabolism, as dietary fat supplementation elevates its concentration. Studies indicate that energy-deficient rice straw diets can trigger body fat mobilization in periparturient cows, increasing serum total cholesterol. Additionally, ketosis in dairy cows is often accompanied by decreased serum total bilirubin. Therefore, elevated serum total cholesterol and reduced total bilirubin in rice straw-fed cows may indicate that low dietary energy prompted body fat mobilization for energy, predisposing to ketosis. Moreover, increased aspartate aminotransferase activity in corn stover-fed cows and elevated total cholesterol in rice straw-fed cows suggest potential hepatic immune stress, which could adversely affect hepatic nutrient metabolism and impair gluconeogenesis. Creatinine, an intermediate product of protein metabolism that indirectly participates in the urea cycle, serves as an important marker of renal dysfunction. Higher serum creatinine in rice straw-fed cows, consistent with the trend for serum urea nitrogen, suggests that elevated serum urea nitrogen increased renal metabolic burden, potentially causing renal dysfunction. Thus, serum indices indicate that replacing alfalfa with straw under conventional forage-to-concentrate ratios may disrupt protein, lipid, and glucose metabolism in liver and kidney, causing functional disturbances that reduce glucose and amino acid supply and impair nitrogen utilization efficiency.

3.3 Impacts on Liver and Kidney Histomorphology

Histological examination revealed normal liver and kidney morphology across all dietary treatments, indicating that despite being low-quality roughages, rice straw and corn stover did not cause direct tissue damage during the 12-week feeding period. However, altered activities of glutathione metabolic enzymes (aspartate aminotransferase and alanine aminotransferase) in serum may indicate a certain degree of oxidative stress. Furthermore, differences in liver and kidney function-related indices between straw-fed and alfalfa-fed cows became more pronounced with prolonged feeding duration. Therefore, longer-term studies are needed to determine whether feeding straw, particularly rice straw, to lactating cows adversely affects health. Additionally, supplementing essential amino acids and other nutrients may improve nitrogen utilization efficiency in straw-based diets, given the low nitrogen efficiency and excessive urea nitrogen excretion.

In conclusion, replacing alfalfa with corn stover or rice straw in dairy cow diets significantly increased fecal and urinary nitrogen excretion, thereby reducing nitrogen utilization efficiency. While no morphological changes were observed in liver and kidney tissues during the experimental period, serum markers reflecting liver and kidney function were significantly elevated, indicating potential metabolic disturbances.

References

[1] 王炳. 饲喂秸秆日粮奶牛泌乳性能低下的消化吸收与代谢机制研究 [D]. 博士学位论文. 杭州: 浙江大学, 2016: 11-12.

[2] 钟华平, 岳燕珍, 樊江文. 中国作物秸秆资源及其利用 [J]. 资源科学, 2003, 25(4): 62-67.

[3] PANG Y Z, LIU Y P, LI X J, et al. Improving biodegradability and biogas production of corn stover through sodium hydroxide solid state pretreatment[J]. Energy & Fuels, 2008, 22(4): 2761-2766.

[4] 毕于运, 高春雨, 王亚静, 等. 中国秸秆资源数量估算 [J]. 农业工程学报, 2009, 25(12): 211-217.

[5] KEBEDE G. Effect of urea-treatment and Leucaena (Leucaena leucocephala) supplementation on the utilization of wheat straw as feed for sheep[D]. Master' s Thesis. Haramaya, Ethiopia: Haramaya University, 2006: 66.

[6] ZHAO T Z, LI H Y. Study on ruminal degradation of mainly protein and fiber sources in dairy diets[J]. Contemporary Animal Husbandry, 2009, 11: 29-32.

[7] WANG B, MAO S Y, YANG H J, et al. Effects of alfalfa and cereal straw as a forage source on nutrient digestibility and lactation performance in lactating dairy cows[J]. Journal of Dairy Science, 2014, 97(12): 7706-7715.

[8] WANG B, SUN H Z, XU N N, et al. Amino acid utilization of lactating

dairy cows when diets are changed from alfalfa-based diet to cereal straw-based diets[J]. Animal Feed Science and Technology, 2016, 217: 56–66.

[9] WANG B, ZHAO F Q, ZHANG B X, et al. An insufficient glucose supply causes reduced lactose synthesis in lactating dairy cows fed straw instead of alfalfa hay[J]. Journal of Animal Science, 2016, 94(11): 4771–4780.

[10] LAPORTE M F, PAQUIN P. Near-infrared analysis of fat, protein, and casein in cow' s milk[J]. Journal of Agricultural and Food Chemistry, 1999, 47(7): 2600–2605.

[11] AOAC. Official methods of analysis[J]. 14th ed. Arlington, VA: Association of Official Analytical Chemists, 1990.

[12] LEE C, HRISTOV A N. Short communication: evaluation of acid-insoluble ash and indigestible neutral detergent fiber as total-tract digestibility markers in dairy cows fed corn silage-based diets[J]. Journal of Dairy Science, 2013, 96(8): 5295–5299.

[13] Statistical Analysis Systems Institute. SAS/STAT® User' s Guide: Statistics. Version 8.1[Z]. 2000.

[14] WOLFE A H, PATZ J A. Reactive nitrogen and human health: acute and long-term implications[J]. AMBIO: A Journal of the Human Environment, 2002, 31(2): 120–125.

[15] KAUFFMAN A J, ST-PIERRE N R. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows[J]. Journal of Dairy Science, 2001, 84(10): 2284–2295.

[16] ZHU W, FU Y, WANG B, et al. Effects of dietary forage sources on rumen microbial protein synthesis and milk performance in early lactating dairy cows[J]. Journal of Dairy Science, 2013, 96(3): 1727–1734.

[17] HUHTANEN P, CABEZAS-GARCIA E H, KRIZSAN S J, et al. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows[J]. Journal of Dairy Science, 2015, 98(5): 3182–3197.

[18] MAHRT A, BURFEIND O, HEUWIESER W. Effects of time and sampling location on concentrations of β-hydroxybutyric acid in dairy cows[J]. Journal of Dairy Science, 2014, 97(1): 291–298.

[19] GRÜNWALDT E G, GUEVARA J C, ESTÉVEZ O R, et al. Biochemical and haematological measurements in beef cattle in Mendoza plain rangelands (Argentina)[J]. Tropical Animal Health and Production, 2005, 37(6): 527–540.

[20] COZZI G, RAVAROTTO L, GOTTARDO F, et al. Short communication: reference values for blood parameters in Holstein dairy cows: effects of parity, stage of lactation, and season of production[J]. Journal of Dairy Science, 2011, 94(8): 3895–3901.

[21] MILLER N E, HAMMETT F, SALTISSI S, et al. Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins[J]. British Medical Journal, 1981, 282(6278): 1741–1744.

[22] PADMAJA K, RAO D S T. Clinical, biochemical and therapeutic responses of Laurabolin® and Ketonex® to post parturient indigestion (PPI) related subclinical ketosis in buffalo[J]. Animal Science Reporter, 2013, 7(1): 35–40.

[23] BERTONI G, TREVISI E. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds[J]. The Veterinary Clinics of North America: Food Animal Practice, 2013, 29(2): 413–431.

[24] ABDELQADER M M, HIPPEN A R, KALSCHEUR K F, et al. Isolipidic additions of fat from corn germ, corn distillers grains, or both on dairy diets[J]. Journal of Dairy Science, 2009, 92(11): 5523–5534.

[25] SORDILLO L M, RAPHAEL W. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders[J]. Veterinary Clinics of North America: Food Animal Practice, 2013, 29(2): 267–278.

[26] GRAUGNARD D E, MOYES K M, TREVISI E, et al. Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge[J]. Journal of Dairy Science, 2013, 96(2): 918–935.

[27] MILES E D, MCBRIDE B W, JIA Y, et al. Glutamine synthetase and alanine transaminase expression are decreased in livers of aged vs. young beef cows and GS can be upregulated by 17β-estradiol implants[J]. Journal of Animal Science, 2015, 93(9): 4500–4509.

[28] ARIAS A, GARCIA-VILLORIA J, RIBES A. Guanidinoacetate and creatine/creatinine levels in controls and patients with urea cycle defects[J]. Molecular Genetics and Metabolism, 2004, 82(3): 220–223.

[29] NUDDA A, CORREDDU F, MARZANO A, et al. Effects of diets containing grape seed, linseed, or both on milk production traits, liver and kidney activities, and immunity of lactating dairy ewes[J]. Journal of Dairy Science, 2015, 98(2): 1157–1166.

[30] ABDEL-WAHHAB M A, NADA S A, KHALIL F A. Physiological and toxicological responses in rats fed aflatoxin-contaminated diet with or without sorbent materials[J]. Animal Feed Science and Technology, 2002, 97(3/4): 209–219.

[31] BERNARDINI D, SEGATO S, MARCHESINI G, et al. Changes in the metabolic profile and performance of dairy cows fed two dietary crude protein concentrations[M]//BOITI C, FERLAZZO A, GAITI A, et al. Trends in Veterinary Sciences. Berlin Heidelberg: Springer, 2013: 125–128.

[32] IQBAL M, SHARMA S D, REZAZADEH H, et al. Glutathione metabolizing enzymes and oxidative stress in ferric nitrilotriacetate mediated hepatic injury[J]. Redox Report, 1996, 2(6): 385-391.

[33] WANG C, LIU H Y, WANG Y M, et al. Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows[J]. Journal of Dairy Science, 2010, 93(8): 3661-3670.

[34] APELO S I A, KNAPP J R, HANIGAN M D. Invited review: current representation and future trends of predicting amino acid utilization in lactating dairy cows[J]. Journal of Dairy Science, 2014, 97(7): 4000-4017.

Note: Figure translations are in progress. See original paper for figures.