Nutritional and Toxicological Assessment of White-Rot Fermented Animal Feed

K. K. Sharma · Bhuvnesh Shrivastava ·
Preeti Nandal · Neeta Sehgal · V. R. B. Sastry ·
A. Kalra · R. C. Kuhad

Received: 10 April 2011 / Accepted: 2 August 2011 / Published online: 13 August 2011
© Association of Microbiologists of India 2011

Abstract The fungal fermented wheat straws as animal feeds have been evaluated for its toxicological and nutritional status in male rats (*Holtzman* strain). Digestibility of dry matter and other nutrients as well as fiber fractions were found significantly higher ($P < 0.05$) in straw fermented with either *Ganoderma* sp. rckk02 (T3) or *Crinipellis* sp. RCK-1 (T4) than unfermented straw (T1) or straw fermented with *Pycnoporus cinnabarinus* (T2). The aflatoxin B1, B2, G1 and G2 were either absent or present in permissive levels in T3 and T4 diets and exhibited normal stress enzyme activity in case of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) enzymes whereas, rats fed on T2 diet showed elevated levels of stress enzymes (ALT, AST and LDH activity), 100% high morbidity and 8.3% mortality. This study suggests that *Ganoderma* sp. rckk02 and

Crinipellis sp. RCK-1 are efficient in improving the nutritive value of poor quality straw and do not possess any threat for their subsequent use as ruminant feed.

Keywords Wheat straw · Solid-state fermentation · White-rot fungi · Aflatoxin

Introduction

Wheat straw is produced in huge quantity worldwide and is commonly used as cattle feed. Over 354.35 trillion grams/year (354×10^9 kg) of wheat straw is produced globally per annum and provides only 3.2% economic return [1, 2]. Like other cereal straws the availability of nutrients from wheat straw is also much lower than the potential energy stored in it. The presence of lignin in wheat straw hinders its fullest use in ruminants.

The straws are not digested efficiently by the enzymes produced by microbial flora and fauna present in the rumen. Various physical, chemical and biological approaches have been constantly attempted to improve the nutritional quality of plant biomass [3–7]. Among microorganisms white-rot fungi, as common decomposer of woody materials are identified as potential lignin degrader, thereby increasing the bio-availability of cellulose and other polymers for microbial fermentation [8–11].

According to various studies lignocellulose biomass fermented with different white-rot fungi such as *Phanerochaete chrysosporium* (ATCC 24725), *Cryptomeria japonica*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Trametes versicolor* and *Ceriporiopsis subvermispora* have been tested for improvement in their digestibility and nutritive value for ruminants [4, 5, 9]. But fungal fermented feed have scarcely been tested for the presence of mycotoxins.

K. K. Sharma · B. Shrivastava · P. Nandal · R. C. Kuhad (✉)
Lignocellulose Biotechnology Laboratory,
Department of Microbiology, University of Delhi South
Campus, New Delhi 110021, India
e-mail: kuhad85@gmail.com

K. K. Sharma
Department of Microbiology, Maharshi Dayanand University,
Rohtak 124001, India

N. Sehgal
Department of Zoology, University of Delhi,
Delhi 110007, India

V. R. B. Sastry
Ganesh Scientific Research Foundation, Kirti Nagar,
New Delhi 110 015, India

A. Kalra
Ayurved Ltd., Sagar Plaza, District Centre, Laxmi Nagar,
Delhi 110092, India

The consumption of mycotoxin-contaminated animal feed may have direct health consequences such as reduced feed intake, feed refusal, poor feed conversion, loss in body weight, increased disease incidence and reduced reproductive capabilities [12, 13]. Thus, it is necessary to evaluate the animal feed for the toxicity and the evaluation is generally done with laboratory animals as direct testing is impractical with bigger animals [14, 15]. Moreover, interest in fermentation with monogastric digestive tract is also growing, particularly relative to animal health.

The objective of this work was to improve the nutritive value of wheat straw by solid-state fermentation with white-rot fungi and to assess their digestibility and toxicity in rats as model system.

Materials and Methods

The methodology has been adopted after considering OECD [16] Guidelines for Testing Acute Oral Toxicity and Igморis Draft, March 2008 and with due approval of institutional ethical committee. The testing has been carried out at Department of Zoology, University of Delhi, Delhi.

Microorganisms and Production of Fungal Fermented Feed

Ganoderma sp. rckk02 and *Crinipellis* sp. RCK-1 were lab cultures and *Pycnoporus cinnabarinus* (ATCC 2004378) was a kind gift from Late Prof. Karl-Erik L. Eriksson, University of Georgia, Athens, USA. The fungal cultures were grown in malt extract medium and inoculum of different fungi were prepared following the method described elsewhere [5]. Enamel trays (25 × 9 × 20 cm²) containing 50 g of wheat straw were moistened with mineral salt solution containing (g l⁻¹): KH₂PO₄, 0.5; MgSO₄·7H₂O, 0.5; Ca(NO₃)₂·4H₂O, 0.5 (pH 5.5) and autoclaved at 121°C for 15 min. Each sterilized tray was inoculated with desired volume of inoculum to obtain 1% (w/w) of fungal dry mass and incubated at 35°C for *Ganoderma* sp. rckk02 and 30°C for *Crinipellis* sp. RCK-1 and *P. cinnabarinus* for 10 days. The uninoculated trays without fungal mycelia were used as control.

Animals and Housing

Forty-eight weaned male rats (*Holtzman* strain) of uniform body weight and 30–32 day old were procured from Department of Zoology, University of Delhi, Delhi and divided into four experimental groups. Each group consisted of three replicates of four rats each in a completely randomized design. The animals were identified uniquely

via ear mark and acclimatized for 5 days on normal diet before start of feeding trial. They were housed in cages fitted with feeders, waterers and with facilities for the collection of faeces. Tap water was used for drinking and supplied *ad libitum*. The temperature and relative humidity of the room was maintained at 25 ± 2°C and 50–55%, respectively.

Chemicals and Feed Materials

All the kits for biochemical assays for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were purchased from Teco Diagnostics, Anaheim, CA 92807. Wheat straw and other feed ingredients were purchased locally.

Feed Composition

The composite diets containing equal parts (50:50) of concentrate ingredients and wheat straw either unfermented (T1) or fermented with different fungi; *P. cinnabarinus* (T2), *Ganoderma* sp. rckk02 (T3) and *Crinipellis* sp. RCK-1 (T4) were pelleted (8 mm diameter) by expander extrusion at a Feed Factory, New Delhi, offered to the animals *ad libitum*. An individual diet was composed of 50% wheat straw (raw or fermented) and 50% concentrate consisting of (%) yellow maize; 10, wheat flour; 10, deoiled soyabean meal; 24, meat cum bone meal; 01, vegetable oil; 2.5, molasses; 1.0, mineral mixture; 1.0 and sea salt; 0.5.

Determination of Body Weights, Feed Efficiency, Dry Matter (DM) Utilization, Nutrient Digestibility and Toxicity Studies

A measured quantity of the respective pelleted composite diets was offered daily morning to the rats in a way to meet *ad libitum* and had access to it at all times. The left over residues were weighed after 24 h throughout the experimental period and before offering fresh feed. Individual body weights of animals were recorded at weekly intervals before offering feed and water. The efficiency of feed and DM utilization was calculated as unit (g) intake per unit (g) gain. A digestibility trial of 4 day duration was conducted on all the experimental rats. The composite diets were analysed commercially for the presence of aflatoxins (B1, B2, G1 and G2) using LC–MS system at Sri Ram Research Institute, Delhi, India.

Analysis of Biological and Blood Samples

Feed and faecal samples were analysed for various proximate principles and fibre fractions [17–19]. Nitrogen in wet

faeces was estimated using CHNS analyzer (ElementarVario EL, Germany) and crude protein was calculated by multiplying nitrogen with 6.25 [17].

At the end of experimental feeding, blood was collected from rats of all the groups through ocular puncture into sterile stoppard glass tubes. Thereafter, plasma was obtained and collected by centrifuging the blood at 4,000 rpm for 5 min and analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) [20, 21].

Statistical Analysis

The data were subjected to analysis of variance as per the methods of Snedecor and Cochran [22].

Results

Chemical Composition and Fibre Fractions of Feeds

Pelleted composite diets were found to be isonitrogenous as crude protein (CP) varied narrowly from 15.17 to 16.95% across the diets. The content of organic matter (OM), ether extract (EE), crude fiber (CF), nitrogen free extractive (NFE), total carbohydrate (T-CHO), acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash varied marginally among diets (Table 1).

Total and Average Body Weight Daily Gain (ADG)

The body weight gain of the rats fed on test diets i.e. *Ganoderma* sp. rckk02 (T3) and *Crinipellis* sp. RCK-1 (T4) showed ADG of 1.593 and 1.393 g, respectively. Whereas, ADG of rats fed on *P. cinnabarinus* fermented wheat straw (T2) was found significantly ($P < 0.01$) reduced. Moreover, comparison at 5% level of significance ($P < 0.05$) revealed that the total as well as ADG were similar on T1 and T3 diets (Table 2).

Feed, Dry matter and Water Intake

The rats fed control diet (T1) consumed significantly ($P < 0.01$) more feed and DM, while those on diet containing *Ganoderma* sp. rckk02 (T3) and *Crinipellis* sp. RCK-1 (T4) responded fairly well. Whereas, *P. cinnabarinus* fermented wheat straw (T2) was consumed less as compared to T3 and T4 test diets and control diet (T1) (Table 2). The daily water intake was lower ($P < 0.05$) in rats fed on T2 diet than other diets. The efficiency of feed utilization (EFU) and dry matter utilization (EDMU) were found maximum in case of T2 diet followed by T4 and T3 diets (Table 2).

Dry Matter and Nutrient Digestibility

The DM of control diet T1 and test diets T3 and T4 was digested significantly ($P < 0.01$) more than the DM of T2 diet having wheat straw fermented with *P. cinnabarinus* (Table 3). The digestibility for OM, CP, EE, CF, NFE and T-CHO was found higher in T3 and T4 diets in comparison with T2 and T1 diets. However, on comparison at lower level of significance ($P < 0.05$), it was found that the rats fed with T3 diet were efficient in digesting OM and T-CHO and rats fed with T4 were superior in digesting EE than rats on other diets (Table 3).

The digestibility of ADF (43.13%) and cellulose (45.02%) was better ($P < 0.01$) on T3 diet than on T4 diet as well as on control diet (T1). The digestibility of NDF ($P < 0.01$) and hemicellulose ($P < 0.05$) on T3 diet was 57.9 and 63.29%, respectively, which is significantly higher than control diet. The digestibility of NDF (28.79%), ADF (20.09%), cellulose (21.98%) and hemicellulose (35.3%) was significantly ($P < 0.01$) depressed on T2 diet compared to the digestibility on control (T1), T3 and T4 diets (Table 3).

The nutritive value of the animal feed was notably improved in both diets fermented with either *Ganoderma* sp. rckk02 (T3) or *Crinipellis* sp. RCK-1 (T4) than unfermented wheat straw (T1) and the diet fermented with *P. cinnabarinus* (T2). The feeding of T3 and T4

Table 1 Proximate principles and chemical composition (% DM) of composite diets

Diet	OM	CP	EE	CF	NFE	T-CHO	ASH	NDF	ADF
T1 (Control)	90.42	16.23	2.36	19.61	52.22	71.83	9.58	48.82	22.16
T2 (<i>P. cinnabarinus</i>)	89.62	15.17	2.51	21.48	50.46	71.94	10.38	55.72	30.01
T3 (<i>Ganoderma</i> sp. rckk02)	92.20	16.69	2.53	24.89	48.09	72.98	7.80	54.70	21.95
T4 (<i>Crinipellis</i> sp. RCK-1)	89.61	16.95	2.84	22.88	46.94	69.82	10.39	56.52	22.03

OM organic matter, CP crude protein, EE ether extract, NFE nitrogen free extractive, T-CHO total carbohydrate, NDF neutral detergent fiber, ADF acid detergent fiber

Table 2 Average daily gain, feed and water intake and efficiency of feed (DM) utilization

Attribute	T1 (Control)	T2 (<i>P. cinnabarinus</i>)	T3 (<i>Ganoderma</i> sp. rckk02)	T4 (<i>Crinipellis</i> sp. RCK-1)	SEM
Initial weight (g)	59.40	59.03	58.73	58.23	0.861
Final weight (g)	111.32 ^A	62.97 ^B	103.39 ^A	97.20 ^A	2.459
Total gain (g)	51.92 ^{Aa}	3.94 ^{Bc}	44.65 ^{Aab}	38.97 ^{Ab}	3.105
Av. daily gain (ADG) (g)	1.857 ^{Aa}	0.137 ^{Bc}	1.593 ^{Aab}	1.393 ^{Ab}	0.111
Av. daily feed intake (g)	12.08 ^{Aa}	3.85 ^{Cd}	7.51 ^{Bc}	8.30 ^{Bb}	0.218
Av. daily DM intake (g)	11.26 ^{Aa}	3.80 ^{Cd}	6.88 ^{Bb}	7.60 ^{Bc}	0.200
Efficiency feed utilization	6.51 ^B	13.17 ^A	4.74 ^C	6.44 ^B	0.245
Efficiency of DM utilization	6.07 ^B	13.07 ^A	4.34 ^C	5.89 ^B	0.242
Av. feed intake/g B.Wt.(g)	0.106 ^{Aa}	0.047 ^{Cc}	0.070 ^{Bbc}	0.080 ^{Bab}	0.0032
Av. DMI/g B.Wt. (g)	0.132 ^{Aa}	0.062 ^{Cd}	0.085 ^{Bc}	0.098 ^{ABb}	0.0029
Av daily water intake (ml g ⁻¹ Body Wt.)	0.397 ^a	0.298 ^c	0.334 ^b	0.374 ^{ab}	0.0143

Means bearing different superscripts (^{ABC} $P < 0.01$; ^{abcd} $P < 0.05$) in a row differ significantly

Table 3 Dry matter and nutrient digestibility (%)

Nutrient	T1 (Control)	T2 (<i>Pycnoporus cinnabarinus</i>)	T3 (<i>Ganoderma</i> sp. rckk02)	T4 (<i>Crinipellis</i> sp. RCK-1)	SEM
Dry matter	52.91 ^A	21.37 ^B	56.18 ^A	53.10 ^A	1.298
Organic matter	54.76 ^{Bb}	27.03 ^{Cc}	58.99 ^{Aa}	55.70 ^{Bab}	0.619
Crude protein	56.10 ^A	22.16 ^B	60.73 ^A	59.83 ^A	2.103
Ether extract	66.92 ^{Bc}	36.32 ^{Cd}	75.75 ^{Ab}	77.96 ^{Aa}	1.390
Crude fibre	47.59 ^A	27.55 ^B	52.01 ^A	48.08 ^A	1.379
Nitrogen free extract	56.49 ^A	27.12 ^B	61.11 ^A	56.58 ^A	1.319
Total carbohydrate	55.06 ^{Ab}	27.25 ^{Bc}	58.01 ^{Aa}	53.79 ^{Ab}	0.740
NDF	50.08 ^B	28.79 ^C	57.90 ^A	53.73 ^{AB}	1.223
ADF	34.84 ^C	20.09 ^D	43.13 ^A	38.96 ^B	0.741
Cellulose	35.32 ^B	21.98 ^C	45.02 ^A	37.92 ^B	0.948
Hemicellulose	53.43 ^{Bb}	35.30 ^{Cc}	63.29 ^{Aa}	60.90 ^{ABa}	1.658

Means bearing different superscripts (^{ABCD} $P < 0.01$; ^{abc} $P < 0.05$) in a row differ significantly

diets resulted in comparatively better absorption of nutrients, which in turn caused a higher digestibility.

Blood Enzyme Activity

The activity (U/L) of ALT and AST varied significantly ($P < 0.01$) due to dietary variations (Table 4). The activity of these enzymes was found to be within the normal physiological range i.e. ALT 35.1 ± 13.3 U/L and AST 42.9 ± 10.1 U/L, in control diet (T1) and test diets i.e. T3 and T4 [23]. The feeding of T2 diet resulted in elevated activity of ALT and AST i.e. 46.08 and 52.56 (U/L), respectively. The LDH activity in rats of all the test diets i.e. T3 and T4 was within the normal reported values of 46.6 ± 22.0 (U/L) (Table 4) [23], whereas, the LDH

activity was significantly ($P < 0.01$) higher on T2 diet (78.31 U/L).

Mycotoxins

Mycotoxins were either absent or present at permissible levels in control (T1), T3 and T4 diets. The diets containing control wheat straw (T1) or fermented with *Ganoderma* sp. rckk02 (T3) and *Crinipellis* sp. RCK-1 (T4) were found to have negligible amount (<3 ppb) of various mycotoxins i.e. aflatoxin B1, B2, G1 and G2. However, the diet containing *P. cinnabarinus* fermented wheat straw (T2) contained more levels of mycotoxins namely aflatoxin B1 (0.67 ppb), B2 (1.74 ppb), G1 (0.69 ppb) and G2 (4.92 ppb) than T1, T3 and T4 diets (Table 5). But the

Table 4 Activity (U/L) of blood enzymes

Enzyme	T1 (Control)	T2 (<i>Pycnoporus cinnabarinus</i>)	T3 (<i>Ganoderma</i> sp. rckk02)	T4 (<i>Crinipellis</i> sp. RCK-1)	SEM
SGPT (ALT)	34.30 ^B	46.08 ^A	36.30 ^B	36.99 ^B	0.679
SGOT (AST)	44.81 ^B	52.56 ^A	42.63 ^B	44.43 ^B	0.936
LDH	51.84 ^C	78.31 ^A	51.76 ^C	61.07 ^B	1.968

Means bearing different superscripts (^{ABC} $P < 0.01$) in a row differ significantly

Table 5 Mycotoxin levels (μg/kg or ppb) in composite pelleted diets

Mycotoxin	T1 (Control)	T2 (<i>P. cinnabarinus</i>)	T3 (<i>Ganoderma</i> sp. rckk02)	T4 (<i>Crinipellis</i> sp. RCK-1)
Aflatoxin B1	ND	0.67	ND	ND
Aflatoxin B2	0.55	1.74	ND	0.37
Aflatoxin G1	0.49	0.69	0.30	0.21
Aflatoxin G2	1.50	4.92	1.57	2.63

observed levels of aflatoxins in all the diets were far below the permissible levels (20 ppb) in the feeds meant for immature animals (USFAD) and poultry (BIS).

Mortality and Morbidity

The rats fed on test diets T3, T4 and T1 were found to be physically more active. But T2 diet showed 100% morbidity as indicated by depressed growth, lowered feed intake and one rat died on final day resulting in 8.3% mortality on T2 diet.

Discussion

Ganoderma sp. rckk02 and *Crinipellis* sp. RCK-1 used in the present study are our own lab cultures. Both cultures luxuriantly colonize the substrate and comparatively degrade more lignin than cellulose, which qualify them to be used for upgrading the animal feed. The fungal fermented feeds have been evaluated for their nutritional and toxicological status in male rats as model system. The prejudice against using female animals may be partly due to the concern that they are intrinsically more variable than males because of cyclical reproductive hormones, making them unsuitable for use as baseline models [24].

The proximate analysis of composite diets revealed that all the nutrients like OM, EE, CF, NFE, T-CHO and ash varied marginally among them, however, EE content of fungal fermented diets was considerably improved, which could be attributed to value addition to diet by fungal fermentation. The observed increase in crude protein and EE could have provided energy in terms of more fermentable carbohydrates and increased digestibility of fermented diets [5]. Similar findings are reported by Akinfemi et al. [25], where increase in digestibility of feedstuff was attributed to the breakdown of CF and ADF content and

increase in CP content. The improved dry matter intake (DMI) in *Ganoderma* sp. rckk02 fermented diet (T3) and *Crinipellis* sp. RCK-1 diet (T4) was probably responsible for higher nutrient availability. Contrary to this, diet fermented with *P. cinnabarinus* (T2) exhibited lower ($P < 0.05$) DMI, consequently causing poor nutrient digestibility.

Biochemical studies of the blood samples showed normal level of stress enzymes in the rats fed on either *Ganoderma* sp. rckk02 fermented diet (T3) or *Crinipellis* sp. RCK-1 fermented diet (T4), whereas elevated levels of ALT, AST and LDH were observed in the rats fed on the diet containing wheat straw fermented with *P. cinnabarinus* (T2).

ALT is commonly measured for evaluation of hepatocellular injury to determine liver health. When the liver is damaged or diseased, it releases ALT into the bloodstream, which makes ALT levels go up [26]. AST is an important enzyme in amino acid metabolism and found in the liver, heart, skeletal muscle, kidneys, brain and red blood cells and it is commonly measured clinically as a marker for liver health. Low levels of AST are normally found in the blood when body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage [26]. LDH is an oxidoreductase, which catalyzes the inter-conversion of lactate and pyruvate and most often measured to check for tissue damage e.g., hemolysis and others disorders i.e. cancer, meningitis, encephalitis, acute pancreatitis and HIV [27]. A typical range is 105–333 IU/L for LDH.

The elevated level of stress enzymes and mycotoxins present in T2 diet may probably have contributed to the observed mortality and morbidity. Moreover, it is possible to induce a toxicological change due to simple alterations of dietary minerals or energy. These changes could be

wrongly ascribed to biologically fermented feed; therefore precaution may be taken while formulation of the test diets as described by Villas-Bôas et al. [15].

Conclusion

Feeding with diet fermented with *Ganoderma* sp. rckk02 (T3) and *Crinipellis* sp. RCK-1 (T4) has improved the nutrient utilization among rats and did not induce any stress on animals and hence appear to be safe for animal feeding.

Acknowledgments The authors gratefully acknowledge the financial assistance from Department of Biotechnology, Government of India, New Delhi and Ayurvet Pvt. Ltd. Delhi. The authors are grateful to Prof. P. N. Bhat, Former D. D. G., Indian Council of Agricultural Research, for all moral support and constructive criticism and suggestions during the course of the study.

References

- Kim S, Dale BE (2004) Global potential bioethanol production from wasted crops and crop residues. *Biomass Bioenerg* 26:361–375
- Hoskinson RL, Hess JR, Foust TD, McKean WT, Lewis MS (2001) Fractionation of higher value crop residue components for conversion into bioenergy and industrial products, ASAE Annual International Meeting, Sacramento, California, USA
- Kuhar S, Nair LM, Kuhad RC (2008) Pretreatment of lignocellulosic material with fungi capable of higher lignin degradation and lower carbohydrate degradation improves substrate acid hydrolysis and the eventual conversion to ethanol. *Can J Microbiol* 54:305–313
- Kumar S, Gomes J (2008) Performance evaluation of reactors designed for bioconversion of wheat straw to animal feed. *Anim Feed Sci Technol* 144:149–166
- Shrivastava B, Thakur S, Khalsa YP, Gupte A, Puniya AK, Kuhad RC (2011) White-rot fungal conversion of wheat straw to energy rich cattle feed. *Biodegradation* 22:823–831
- Sahoo B, Sarawat ML, Haque N, Khan MY (2002) Influence of chemical treatment of wheat straw on carbon, nitrogen and energy balance in sheep. *Small Rumin Res* 44:201–206
- Okano K, Ohkoshi N, Nishiyama A, Usagawa T, Kitagawa M (2009) Improving the nutritive value of madake bamboo, *Phyllostachys bambusoides*, for ruminants by culturing with the white-rot fungus *Ceriporiopsis subvermisporea*. *Anim Feed Sci Technol* 152(3):278–285
- Kirk TK, Farrel RL (1987) Enzymatic combustion: the microbial degradation of lignin. *Ann Rev Microbiol* 41:465–505
- Akin DE, Sethuraman A, Morrison WH, Martin SA, Eriksson KEL (1993) Microbial delignification with white rot fungi improves forage digestibility. *Appl Environ Microbiol* 59(12):4274–4282
- Kuhad RC, Singh A, Eriksson KEL (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. *Adv Biochem Eng Biotechnol* 57:47–125
- Sharma RK, Arora DS (2010) Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by *Phlebia floridensis*. *Biore-sour Technol* 101:9248–9253
- Morgavi DP, Riley RT (2007) An historical overview of field disease outbreaks known or suspected to be caused by consumption of feed contaminated with Fusarium toxins. *Anim Feed Sci Technol* 137:201–212
- Binder EM, Tan LM, Chin LJ, Handl J, Richard J (2007) Worldwide occurrence of mycotoxins in commodities, feed and feed ingredients. *Anim Feed Sci and Technol* 137:265–282
- Hoogerheide JC, Yamada K, Littlehales JD, Ohno K (1979) Guidelines for testing of single cell protein destined as protein source for animal feed (II). *IUPAC* 51:2537–2560
- Villas-Bôas SG, Esposito E, Mitchell DA (2002) Microbial conversion of lignocellulosic residues for the production of animal feeds. *Anim Feed Sci Technol* 98:1–12
- OECD guidelines for testing of chemicals, No. 425. Acute oral toxicity—modified up and down procedure. Organisation for economic cooperation and development, Paris
- AOAC (2000) Association of official analytical chemists (AOAC), official methods of analysis, 17th edn. AOAC, Arlington
- Goering HK, Van Soest PJ (1970) Forage fibre analysis (apparatus, reagents, procedures and some applications). *Agriculture Hand book* 379. ARS, USDA, Washington
- Van Soest PJ, Robertson JB, Lewis BA (1991) Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74:3583–3597
- Henry JB (2006) Clinical diagnosis and management by laboratory methods, 21st edn. Saunders Elsevier, Philadelphia
- Babson AL, Babson SR (1973) Kinetic colorimetric measurement of serum lactate dehydrogenase activity. *Clin Chem* 19(7):766–769
- Snedecor GW, Cochran WG (1989) Statistical methods, 8th edn. Iowa State University Press, Ames
- Kaneko JJ, Harvey JW, Bruss ML (1997) Clinical biochemistry of domestic animals, 5th edn. Academic Press, New York, pp 895–899
- Zucker I, Beery AK (2010) Males still dominate animal studies. *Nature* 46:690
- Akinfemi A, Mohamed MI, Ayoade JA (2009) Biodegradation of cowpea shells by *Pleurotus* species for its use as ruminant feed. *World J Agric Sci* 5(5):639–645
- Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) (2007). Technical Bulletin 114, 3347. Investment Blvd. Hayward, CA 94545 U.S.A. Cholestech Corp. MKT12916 Rev. B
- Coley HM, Lewandowicz G, Sargent JM, Verrill MW (1997) Chemosensitivity testing of fresh and continuous tumor cell cultures using lactate dehydrogenase. *Anticancer Res* 17(1A):231–236