Efficacy of Glucomannan-containing Yeast Product (Mycosorb®) and Hydrated Sodium Calcium Aluminosilicate in Preventing the Individual and Combined Toxicity of Aflatoxin and T-2 Toxin in Commercial Broilers

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ABSTRACT : A feeding trial was conducted on commercial broilers for a period of 35 days to determine the individual and combined effects of aflatoxin (AF) and T-2 toxin (T-2) on performance, organ weights and immune status. The efficacy of dietary glucomannan-containing yeast product (GYP) (Mycosorb®) and hydrated sodium calcium aluminosilicate (HSCAS) in preventing the adverse effects of aflatoxin and T-2 toxin was also evaluated. Twelve dietary treatments (4×3 factorial) comprising two dietary levels each of AF (0 and 2 mg/kg), T-2 toxin (0 and 1 mg/kg), GYP (0 and 1 kg/ton) and HSCAS (0 and 10 kg/ton) were tested on 720 commercial broiler chickens divided at random into 36 replicates of 20 chicks each (10 males and 10 females). Weight gain and feed intake were recorded weekly. Organ morphology and antibody titers for Newcastle disease (ND) and infectious bursal disease (IBD) were measured on the 35th day. AF and T-2 toxin individually decreased weight gain and increased feed conversion ratio (FCR) ($p<0.05$). AF alone ($p<0.05$) increased weights of liver, kidney, gizzard and spleen and reduced thymus and bursal weights. T-2 toxin ($p<0.05$) increased liver and gizzard weights and decreased thymus weight. Both AF and T-2 toxin when fed individually affected ND and IBD titers in a significant manner. Significant interactions between AF and T-2 toxin were observed for their additive effects on weight gain, FCR, organ weights and antibody titers. Addition of GYP ($p<0.05$) improved weight gain, feed conversion efficiency and restored the organ weights. Antibody titers against ND and IBD were significantly improved with the supplementation of GYP. Supplementation of HSCAS ($p<0.05$) resulted in improvement in weight gain and restored organ weights in the groups fed AF alone, but not in T-2 toxin fed groups. HSCAS inclusion did not influence FCR in toxin fed groups. Addition of HSCAS ($p<0.05$) improved the antibody titers against ND and IBD only in AF fed groups. Thus, the results indicate that addition of GYP is effective in averting the individual and combined toxicity of aflatoxin and T-2 toxin in commercial broilers, while HSCAS is effective only against aflatoxin. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 877-883)***

Key Words : Glucomannan-containing Yeast Product, HSCAS, Aflatoxin, T-2 Toxin, Broiler Chickens

INTRODUCTION

Among the different known mycotoxins, aflatoxin, ochratoxin and T-2 toxin pose significant threat to poultry and are frequently encountered in animal feeds (Devegowda et al., 1998a). Among aflatoxins (AF), AFB_1, a metabolite of *Asperigillus flavus* and *Asperigillus parasiticus*, is an extremely hepatoxic (Rizzi et al., 1998) and carcinogenic compound (Sengstag, 1997). AF causes a variety of effects in poultry including poor performance, altered organ morphology, serum biochemistry and haematology (Raju and Devegowda, 2000; Afzal and Saleem, 2004).

T-2 toxin (T-2) is produced by *Fusarium sporotrichiodes* and considered to posses the highest relative toxicity among the 16 trichothecenes studied (Atroshi et al., 2002). It has been reported to cause decreased weight gain, feed intake and antibody titers against Newcastle Disease and infectious bursal disease and oral lesions, abnormal behaviour, altered feathering and coagulopathy in broiler chickens (Hoerr et al., 1982; Huff et al., 1988; Raju and Devegowda, 2000).

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Received February 22, 2005; Accepted August 22, 2005

The co-occurrence of aflatoxin and T-2 in a single feedstuff is not unlikely (Chandrasekaran, 1996) as feedstuffs are exposed to a variety of climatic conditions in the field and during transit and storage. Huff et al. (1988) observed a significant synergistic interaction between AF and T-2 toxin on several parameters of growing broiler chicks. In many cases, these mycotoxins can be found in combination in contaminated feed with additive toxic effects (Raju and Devegowda, 2000).

Extensive research has been conducted to prevent mycotoxicoses that mainly include physical, chemical, nutritional or biological approaches. The use of adsorbing agents, which can trap the mycotoxin molecule by means of ion exchange and thereby hindering their absorption into blood from the gastrointestinal tract, has gained much attention in prevention of mycotoxins. Hydrated sodium calcium aluminosilicate (HSCAS) (Kubena et al., 1990; Huff et al., 1992; Jindal et al., 1993), bentonite (Santurio et al., 1999), zeolite ore compounds (Harvey et al., 1993), spent canola oil bleaching clays (Smith, 1984), activated charcoal (Edrington et al., 1997), inorganic sorbents (Bailey et al., 1998) and a blend of organic acids and aluminosilicates (Mahesh and Devegowda, 1996) have shown considerable promise in preventing aflatoxicosis.

These clays, however, have some disadvantages like high inclusion rates (Devegowda et al., 1998b), possible interaction with the essential nutrients (Moshtaghian et al., 1991) and lack of binding effect against many mycotoxins of practical importance (Chestnut et al., 1992). Aluminosilicates are reported to selectively bind only those mycotoxin molecules that have polar function atomic groups.

Advances in the field of biotechnology have opened a new avenue for tackling mycotoxicoses. A natural organic product, glucomannan-containing yeast product (GYP), a cell wall derivative of *Saccharomyces cerevisiae* [1026], *have* shown considerable binding ability with several commonly occurring mycotoxins (Devegowda and Murthy, 2005) and is found beneficial in minimizing the adverse effects of mycotoxins in livestock and poultry (Raju and Devegowda, 2000; Whitlow et al., 2000; Dvorska and Surai, 2001; Swamy et al., 2002; Arvind et al., 2003; Murthy and Devegowda, 2004). The present trial was conducted to evaluate the efficacy of glucomannan-containing yeast product and HSCAS on performance, organ morphology and serum immunological variables in broiler chickens exposed to individual and combined mycotoxicoses of aflatoxin B_1 and T-2 toxin.

MATERIALS AND METHODS

Production and quantification of mycotoxins

Aflatoxin B_1 and T-2 toxin were produced employing solid substrate fermentation by methods of Shotwell et al. (1966) and Burmeister (1971), respectively. The fungal cultures used were *A. parasiticus* NRRL 2999 (source: National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 61604, USA) and *F. sporotrichiodes* MTCC 1894 (source: Institute of Microbial Technology, Chandigarh 160 036, India) for aflatoxin B_1 and T-2 toxin, respectively. Mycotoxin content of the culture material was estimated by thin layer chromatography by the method of the Association of Official Analytical Chemists (1995) for AF, and Rukmini and Bhat (1978) and Romer et al. (1978) for T-2 toxin. The AF and T-2 contents were 300 and 100 mg/kg, respectively.

Animal and diets

720 commercial broilers, 1-day-old sexed, were divided at random into 36 replicates of 20 chicks each having equal number of males and females. Temperature and lighting regimen were in accordance with recommendation of the commercial broiler chickens. Feed and water were provided *ad libitum*. Twelve dietary treatments were prepared in a 4 x 3 factorial arrangement with mycotoxin groups (0, AF, T-2, AF+T-2) and mycotoxin binder groups (0, GYP, HSCAS) as main factors. The dietary inclusion levels of AF, T-2, GYP (Mycosorb®) (A proprietary product of Alltech Inc., Nicholasville, KY, USA) and HSCAS were 2 mg/kg, 1 mg/kg, 1 kg/ton and 10 kg/ton, respectively. A corn-soya basal diet was formulated to meet the nutrient requirements as per the specifications of BIS (1992). The starter diet was fed from 0 to 3 weeks (Corn, 63 kg; Soybean, 34 kg; Mineral mixture, 3.2 kg; Salt, 0.3 kg with other feed additives to make up 20.84% crude protein, 2.67% crude fat, 3.88% crude fibre and 2,895 kcal ME/kg) and the finisher diet from 4 to 5 weeks (Corn, 69 kg; Soybean, 28 kg; Mineral mixture, 3.2 kg; Salt, 0.3 kg, with other feed additives to make up 18.58% crude protein, 2.84% crude fat, 3.71% crude fibre and 2,994 kcal ME/kg). Feed ingredients used in formulating the control diet did not contain mycotoxins at detectable levels. The required quantities of culture materials of AF and T-2, GYP and HSCAS were added to the basal diet to prepare different experimental diets. Each diet was fed *ad libitum* to each treatment of three replicate groups for 35 days.

Data collection

Performance : Chicks were weighed individually, and feed consumption for each pen was measured weekly during the 5-wk experiment. Cumulative weight gain and feed consumption were determined, whereas weekly and cumulative gain:feed ratios were calculated. Feed consumption and gain:feed was adjusted for mortalities when appropriate.

Organ morphology : At 35 days of age, 6 birds (3 males and 3 females) in each treatment were humanely euthanised. Weights of liver, kidney, gizzard, spleen, bursa of Fabricius and thymus were recorded. The weights were adjusted to 1kg live weight and means were calculated.

Immunological parameters : Blood was collected at 5 wk of age from 6 birds (3 males and 3 females) in each treatment in non-heparinised tubes by brachial vein puncture. Serum was collected and antibody titers against ND and IBD were determined employing ELISA technique using commercial test kits (Source: Kirkegaard and Perry Laboratories, Gaithersburg, Maryland 20879, USA). The plates were read at 405 on ELISA reader (Labsystem Multiscan MS, Labsystem, S00881 Helsinki, Finland).

Statistical analysis

Data were subjected to analysis of variance by the GLM procedure of SAS® (SAS Institute, 1996). The differences in means were compared using Duncan's multiple range test. Statements of statistical significance were based on $p<0.05$.

RESULTS

Body weight was significantly ($p<0.05$) lowered by both AF and T-2 (Table 1). Combined feeding of AF and T-2

Table 1. Effect of individual and combined toxicity of AF and T-2 with (+) and without (-) GYP and HSCAS on performance of commercial broiler chickens at 35 days of age

Mycotoxin	GYP (1 kg/ton)	HSCAS (10 kg/ton)	Body wt (g)	Feed intake (g/bird)	FCR
-	-	-	1,495^{ab}	2,795^{a}	1.86^{fg}
-	+	-	1,511^{a}	2,783^{abc}	1.84^{g}
-	-	+	1,497^{ab}	2,787^{abc}	1.86^{fg}
AF	-	-	1,311^{d}	2,699^{e}	2.06^{bc}
AF	+	-	1,398^{c}	2,765^{abcd}	1.97^{de}
AF	-	+	1,373^{c}	2,737^{bcde}	1.99^{cde}
T-2	-	-	1,390^{c}	2,735^{cde}	1.96^{de}
T-2	+	-	1,453^{b}	2,793^{ab}	1.92^{ef}
T-2	-	+	1,398^{c}	2,739^{abcde}	1.96^{ed}
AF+T-2	-	-	1,253^{e}	2,693^{e}	2.14^{a}
AF+T-2	+	-	1,361^{c}	2,740^{abcd}	2.09^{bcd}
AF+T-2	-	+	1,311^{d}	2,720^{de}	2.14^{ab}
SEM			5.66	7.28	0.010

AF: 2 mg/kg, T-2: 1 mg/kg.

$^{a-g}$ Means within each column followed by common superscript do not differ significantly ($p \leq 0.05$).

Table 2. Effect of individual and combined effects of AF and T-2 with (+) or without (-) GYP and HSCAS on organ weights (g/kg live wt) at 35 days

Mycotoxin	GYP (1 kg/ton)	HSCAS (10 kg/ton)	Liver	Kidney	Gizzard	Spleen	Thymus	Bursa
-	-	-	27.53^{ed}	8.06^{e}	23.29^{d}	2.0^{c}	3.77^{b}	3.25^{ab}
-	+	-	26.75^{e}	8.17^{e}	23.26^{d}	2.05^{c}	4.15^{a}	3.40^{a}
-	-	+	28.01^{de}	8.21^{e}	23.37^{d}	2.06^{c}	4.29^{a}	3.54^{a}
AF	-	-	33.51^{ab}	10.19^{bc}	27.25^{a}	3.02^{a}	2.89^{e}	2.55^{c}
AF	+	-	29.88^{cd}	9.46^{d}	23.51^{d}	2.03^{c}	3.71^{b}	3.39^{a}
AF	-	+	30.97^{bc}	9.63^{d}	24.50^{c}	2.01^{c}	3.65^{b}	3.42^{a}
T-2	-	-	33.06^{ab}	8.09^{e}	25.81^{b}	1.95^{c}	3.07^{de}	3.15^{ab}
T-2	+	-	27.97^{de}	8.20^{e}	23.28^{d}	2.05^{c}	3.61^{bc}	3.15^{ab}
T-2	-	+	33.04^{ab}	8.21^{e}	25.65^{b}	2.02^{c}	3.15^{de}	3.44^{a}
AF+T-2	-	-	35.16^{a}	11.84^{a}	27.52^{a}	3.06^{a}	2.04^{f}	2.27^{c}
AF+T-2	+	-	31.81^{bc}	9.94^{cd}	23.78^{cd}	2.17^{bc}	3.30^{cd}	3.45^{a}
AF+T-2	-	+	33.00^{ab}	10.71^{b}	26.14^{b}	2.34^{b}	3.14^{de}	2.97^{b}
SEM			0.40	0.16	0.20	0.05	0.07	0.55

AF: 2 mg/kg, T-2: 1 mg/kg.

$^{a-e}$ Means within each column bearing common superscript do not differ significantly ($p \leq 0.05$).

resulted in significant interaction for their additive effects on body weight gain compared to feeding of an individual mycotoxin. The cumulative feed intake was depressed ($p<0.05$) the most by combined feeding of AF and T-2 (3.7%) followed by AF alone (3.4%) and T-2 alone (2.1%). FCR was increased ($p<0.05$) the most by feeding AF and T-2 in combination followed by AF alone and T-2 alone.

Supplementation of GYP to the mycotoxin-contaminated diets significantly alleviated the growth depression effects (Table 1). Addition of GYP significantly improved 5th wk body weight by 6.6%, 4.5% and 8.6% in AF, T-2 and AF+T-2 fed groups respectively. Supplementation of GYP significantly improved the cumulative feed consumption and decreased FCR in all the mycotoxin-fed groups.

Supplementation of HSCAS resulted in improvement ($p<0.05$) of body weight (4.7%), feed intake and FCR in the groups fed AF alone. HSCAS also improved ($p<0.05$) body weight gain by 4.6% in AF+T-2 fed groups but did not influence the feed intake and FCR. HSCAS inclusion showed no effect on groups fed only T-2.

Significant differences were found in the relative weights of liver, kidney, gizzard, thymus and bursa of Fabricius among the treatments (Table 2). AF caused significant increase in size of liver, kidney, spleen and gizzard (21.7%, 26.4%, 51% and 16.8% respectively). T-2 resulted in statistical increase in weights of only liver and gizzard (20 and 10.8%). Significant additive interaction was recorded for the weights of liver, kidney, spleen and gizzard in the groups fed combined mycotoxins.

GYP supplementation significantly reduced the increase in the relative weights of liver, kidney, spleen and gizzard in the groups fed both individual and combined toxins when compared to the unsupplemented control groups (Table 2), whereas supplementation of HSCAS significantly prevented the toxicity in the groups fed AF alone and AF+T-2

Table 3. Effect of individual and combined effects of AF and T-2 with (+) or without (-) GYP and HSCAS on antibody titers in broiler ckickens at week 5

Experimental diets			Antibody (ELISA) titer	
Mycotoxin	GYP (1 kg/ton)	HSCAS (10 kg/ton)	IBD	ND
-	-	-	4,432[b]	4,315[ab]
-	+	-	4,847[a]	4,527[a]
-	-	+	4,317[bc]	4,079[b]
AF	-	-	3,346[ef]	3,282[d]
AF	+	-	4,113[cd]	4,171[b]
AF	-	+	3,976[cd]	4,082[b]
T-2	-	-	3,278[f]	3,681[c]
T-2	+	-	3,955[cd]	4,273[ab]
T-2	-	+	3,222[f]	3,625[c]
AF+T-2	-	-	3,174[f]	3,585[c]
AF+T-2	+	-	3,750[ed]	4,257[ab]
AF+T-2	-	+	3,236[f]	3,636[c]
SEM			93.81	65.76

AF: 2 mg/kg, T-2: 1 mg/kg.

[a-f] Means within each column bearing common superscript do not differ significantly ($p \leq 0.05$).

combination.

Relative weight of bursa was significantly depressed in the AF (30%) and AF+T-2 fed group (30.1%). T-2 showed no effect on relative weight of bursa. The weight of thymus was significantly reduced in the groups fed AF (23.3%), T-2 (18.5%) and AF+T-2 (18.5%). Addition of GYP to diets containing toxins restored the weights of thymus and bursa of Fabricius. Supplementation of HSCAS restored the weights of thymus and bursa in the groups fed AF and AF+T-2, but not in T-2 fed groups.

Antibody titers against ND and IBD were significantly ($p<0.05$) decreased in all the mycotoxin fed groups at 5th wk. Both mycotoxins significantly ($p<0.05$) depressed antibody titers against both diseases (Table 3). Combined feeding of mycotoxins showed further reduction in the titers compared to the feeding of individual mycotoxin against IBD. Lowest titer against ND was seen in AF fed groups. Supplementation of GYP significantly improved antibody titer against both ND and IBD in all the mycotoxins fed groups, while supplementation of HSCAS showed improvement ($p<0.05$) against antibody titers only in the AF fed group.

DISCUSSION

Body weight was depressed in both groups fed individual and combined mycotoxins. Combined feeding of AF and T-2 showed the greatest negative effect. Broiler chickens appear to be highly sensitive to combined toxicity of AF and T-2 compared to individual mycotoxin feeding (Raju and Devegowda, 2000). The increased growth depression observed with the simultaneous feeding of more than one mycotoxin may be due to additive toxic effects of individual toxins (Kubena et al., 1989). Similarly the additive effects of combined feeding of these mycotoxins have been reported by Kubena et al. (1990) and Huff et al. (1992).

A significant interaction was also seen between two mycotoxins for their effects on feed intake. The decreased feed consumption during combined mycotoxicoses has been reported by Kubena et al. (1997a) and Raju and Devegowda (2000).

Feed conversion efficiency was depressed by both the mycotoxins. The lower FCR was noted with these mycotoxins seems to have been mediated through decreased nutrient utilization. Raju and Devegowda (2000) reported additive interaction effects between AF and T-2 in broilers. Nelson et al. (1982) and Johri et al. (1996) recorded decreased amino acid and dry matter digestibility and energy utilization in broiler fed mycotoxins.

Addition of GYP to diets containing toxins significantly improved the performance of broilers. The beneficial effects of GYP on performance of broiler have been reported earlier by Raju and Devegowda (2000); Swamy et al. (2002) and Arvind et al. (2003). These beneficial effects can be attributed to its ability to trap the mycotoxins irreversibly (Devegowda et al., 1996).

HSCAS supplementation improved ($p<0.05$) the performance of broilers in the AF fed groups. These findings are in agreement with previous results of the protective effects of HSCAS compound (Ledoux et al., 1999). Addition of HSCAS improved the body weight in groups fed combined toxins, but did not influence the feed intake and FCR. This beneficial effect of HSCAS on combined toxicity may be attributed to the preventive effect of HSCAS on aflatoxin alone toxicity. The results of the present study are in accordance with earlier study of Kubena et al. (1990). A proposed mechanism of AF chemisorptions by HSCAS is the formation of a complex by the B carbonyl system of the AF with uncoordinated “edge site “ aluminium ions in HSCAS allowing the mycotoxin to pass harmlessly through the animal (Phillips et al., 1990a).

The addition of HSCAS at the rate 10 kg/ton of diet did not afford any protection against toxicity of T-2 on any of the performance parameters. Previous work of Kubena et al. (1998) and Chestnut et al. (1992), supported the current findings.

The weights of liver, kidney and gizzard were increased ($p<0.05$) by feeding AF and AF+T-2. AF B_1 being highly hepatotoxic brings about appreciable changes in the general functioning and gross appearance of liver. The current results support the earlier work of Kubena et al. (1998) for increase of the organ weights.

T-2 increased the relative weights of liver and gizzard. The results are in accordance with findings of Kubena et al. (1990) and Arvind et al. (2003) for the increase in the

weight of gizzard but the increase in the weight of the liver by T-2 have not been previously reported and it was contrast to the earlier studies. In previous works, higher doses were applied, and some other effects were seen, such as oral ulcers, which did not appear in the birds treated with T-2 in this research, likely due to dose and time of exposure. The increase in the relative weight of gizzard may be due to the results of severe inflammation and thickening of mucosal layer. The lack of any significant effect of T-2 on the weight of kidney and spleen observed in this study is similar to the findings of Kubena et al. (1997b, 1998) and Raju and Devegowda (2000).

A significant additive interaction between AF and T-2 was noticed for organ weights. One of the proposed mechanism for aflatoxin toxicity is expressed through the disruption of protein synthesis by a 2, 3 epoxide binding to DNA and inhibiting RNA synthesis (Yu, 1981). T-2 toxic effects have been described as radiomimetic and also inhibit protein synthesis through the inactivation of initiation and termination, possibly through its binding to ribosomes (Ueno, 1977). Therefore, aflatoxin primarily effects protein synthesis during transcription, and T-2 toxin primarily effects protein synthesis during translation which may partially account for their synergistic toxicity.

The mode of action of GYP in decreasing the organs weights is not clear. It is thought to trap the mycotoxin molecule in its glucomannan matrix, which prevents its absorption from gastrointestinal tract and the subsequent toxin-induced tissue changes. The reductions caused by HSCAS in the organ weights were earlier reported by Kubena et al. (1998). The basic mechanism for protection against the toxicity of AF appears to involve sequestration of AF in the gastrointestinal tract and chemisorption of AF (Phillips et al., 1990b).

Relative weights of thymus and bursa of Fabricius were significantly affected by dietary treatments. Atrophy of the lymphoid organs was also reported earlier during mycotoxicoses (Dwivedi and Burns, 1984; Devegowda et al., 1995) and are in line with results of the present trial. The reductions in size of these organs might have been due to necrosis and cellular depletion by the mycotoxins (Hoerr et al., 1981). The two mycotoxins exerted potentiated depressing effects on bursa and thymus weight when fed in combination than in isolation, suggesting additive toxic effects among them on lymphoid organs (thymus and bursa). Similar results were reported by Raju and Devegowda (2002).

Supplementation of GYP to the toxin diets significantly improved the weights of thymus and bursa of Fabricius. Swamy and Devegowda (1998) and Raju and Devegowda (2002) earlier reported improvement in the weight of lymphoid organs with the supplementation of GYP. HSCAS was also found effective in counteracting adverse effects of AF on thymus and bursal weights. The ineffectiveness of HSCAS to counteract the toxicity of T-2 was earlier reported by Chestnut et al. (1992) and Kubena et al. (1998).

Determination of serum titers to ND and IBD after regular vaccination is a method, which is often used to evaluate immunomodulating effects of certain mycotoxins *in vivo*. Antibody titers against ND and IBD were significantly ($p<0.05$) decreased in all the mycotoxin fed groups (individual and combined). Devegowda et al. (1995), Swamy and Devegowda (1998) and Raju and Devegowda (2002) reported the reduction in antibody titers during mycotoxicoses. GYP significantly improved antibody titers against both ND and IBD. Similar improvements in immune response with mannanoligosaccharide supplementation were recorded earlier (Savage et al., 1996). This might have been due to its mycotoxin binding ability and for its indirect effects on cellular immunity through activation of B cells, T cells and macrophages (Lyons, 1994). It has been reported that GYP significantly improve antibody levels in broiler chickens, fed graded levels of AF (Swamy and Devegowda, 1998), multiple mycotoxins (Raju and Devegowda, 2002) and also it inhibited lipid peroxidation in liver of quails fed T-2 toxin (Dvorska and Surai, 2001). Its mycotoxin binding ability might have been primarily responsible for these beneficial effects noted on immune competence. HSCAS inclusion to AF diets improved ($p<0.05$) the ND and IBD titers and it is in accordance with Barmase et al. (1990).

The results thus indicated additive or synergistic effects between the two tested mycotoxins on some of the variables. Glucomannan-containing yeast product (Mycosorb®) improved body weight and feed intake, restored the organ weights and improved antibody titers, thus preventing the individual and combined toxicity of aflatoxin and T-2 toxin in commercial broilers, while HSCAS was only beneficial against aflatoxin.

ACKNOWLEDGEMENT

The authors acknowledge M/S Alltech Inc, USA for providing the financial support and glucomannan-containing yeast product required for this study.

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