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A Meta-analysis of Bentonite Efficacy on Performance, Carcass Yield, Giblet, and Blood Constituents of Broiler Fed Contaminated Aflatoxin

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ABSTRACT

Aflatoxins can easily grow and develop in many feed ingredients and influence the risk of several animal chronic diseases. The present study aimed to evaluate the effect of bentonite inclusion on performances, carcass yield, giblet, and blood constituents of broiler-fed contaminated aflatoxin through a meta-analytical approach. A database was developed based on scientific publications that were searched using several search tools such as Science Direct, PubMed, Scopus, and Google Scholar with “bentonite”, “clay”, “montmorillonite”, “aflatoxin” and “broiler” as keywords. A total of 31 studies were retrieved and included in the analysis. Data analysis was based on the mixed model in which dietary bentonite inclusion was treated as the fixed effect and different studies were considered as random effects. Bentonite used were sodium bentonite, calcium bentonite, and montmorillonite form with levels ranging from 0 to 5%. Meanwhile, aflatoxin B1 levels in the diet ranged from 0 to 5 mg/kg. In this study, body weight gain, feed intake, and feed efficiency showed a linear increase ($p<0.001$) in all phases. The mortality rate linearly decreased ($p<0.05$) in the cumulative. Bentonite inclusion linearly increased ($p<0.001$) the carcass, breast, leg, bursa of Fabricius, and gizzard weight proportion. The percentage of kidney, liver, heart, pancreas, uric acid, and gamma-glutamyltransferase linearly decreased ($p<0.001$) by increasing bentonite levels. Dietary bentonite linearly increased ($p<0.001$) total protein, albumin, aspartate aminotransferase, calcium, glycogen, globulin, and hemoglobin ($p<0.05$). It was concluded that bentonites have the ability to eliminate the detrimental effects of aflatoxin, which may increase the broiler performance, carcass yield and prevent the change in abnormal giblet weight. For effectiveness and animal safety, the EFSA recommends using a maximum of 2% bentonite in the feed.

Keywords: Aflatoxin, Bentonite, Body weight gain, Feed efficiency, Mixed model, Montmorillonite

1. Introduction

Aflatoxins are secondary metabolites produced by *Aspergillus* species that can easily grow and develop in many feed ingredients and influence the risk of several animal chronic diseases. Aflatoxin B1 is the most toxic among the aflatoxins types such as aflatoxin B2, aflatoxin G1, and aflatoxin G2 (Manafi 2012). Acute aflatoxicosis in broilers occurs in excessive and long-term consumption of aflatoxin, and it has effects such as fatigue, anorexia, anemia, bleeding, respiratory distress, pubescence, bloody diarrhea, and high mortality in poultry (Mohamadi & Alizadeh 2010). In low levels and long-term consumption, aflatoxin causes poor performance in poultry and a decrease in egg production, feed consumption, and egg weight (Nasaruddin et al. 2021). In the poultry industry, aflatoxins cause serious economic losses due to these negative effects (Bryden 2012). Therefore, effective methods are urgently needed for the detoxification of feeds containing aflatoxin.

For this purpose, some adsorbents are used in the poultry diet to reduce their absorption from the digestive system by binding aflatoxin. Some adsorbents have been tested *in vitro* as well as *in vivo*, including bentonites. The main advantages of using bentonite as an adsorbent in the feed are its affordability, safety, and convenience. Bentonite belongs to the smectite family, which is mostly composed of montmorillonite clay (Pouraboulghasem et al. 2016). Bentonite primarily contains sodium montmorillonite or calcium montmorillonite, which sodium montmorillonite can absorb huge amounts of water and has greater swelling potential than calcium montmorillonite (Park et al. 2016). It has been reported that bentonite has been found effective in counteracting mycotoxins (Jaynes & Zartman 2011), and improves broiler weight gain (Ani et al. 2014) and egg production (Darmawan & Ozturk 2022). Dietary bentonite also increased feed efficiency but did not affect blood constituents and carcass yield (Khanedar et al. 2013). However, a meta-analysis study that can describe quantitatively summarize the effectiveness of dietary bentonite on broiler has not been provided yet. Therefore, our study aimed to evaluate the bentonite influence on performances, carcass yield, percentage of giblet, and blood constituents of broiler-fed aflatoxin-contaminated feed using data from published articles in a meta-analysis study. It was hypothesized that bentonite would eliminate the negative effects of aflatoxin by binding the mycotoxin in the digestive tract and it would improve broiler performance.

2. Material and Methods

2.1. Development of the database

The journal's search engines (Web of Science, Science Direct, Scopus, and Pub Med) were used to find articles that discussed the different dosages used of bentonite in broiler diets (Table 1). "Bentonite", "clay", "montmorillonite", "aflatoxin", and "broiler" were used as keywords. The criteria article used to be included into the database were: (a) conducting *in vivo* trials on broiler chicken, (b) adding bentonite to basal diets, (c) containing aflatoxin as positive control and/or without aflatoxin as negative control treatments (d) reporting broiler performances, carcass yield, percentage of giblet, blood parameter, (e) the data were not presented in the graph, and (f) articles in English. A total of 100 studies were found from the electronic database. Thus, the studies were screened based on their title and abstract, with 32 deleted related to unsuitable articles such as duplicate articles, review articles, data in graph form, and articles not written in English. Finally, 31 studies were fully reviewed and included in the analysis.

Table 1- Description of the studies included in the database

Authors	*Birds (n)*	*Bentonite Form*	*Dosage (%)*	*Aflatoxin Level (mg/kg)*	*Period (day)*
Tauqir et al. (2001)	240	Sodium bentonite	0-4.5	0	1-42
Rosa et al. (2001)	100	Sodium bentonite	0-0.3	0-5	30-52
Xia et al. (2004)	240	Montmorillonite	0-0.15	0	1-49
Miazzo et al. (2005)	160	Sodium bentonite	0-0.3	0-2.5	23-50
Thalij (2005)	100	Sodium bentonite	0-0.5	0-2.5	1-21
Bailey et al. (2006)	900	Montmorillonite	0-0.5	0-4	1-42
Shi et al. (2006)	160	Montmorillonite	0-0.3	0-0.1	1-42
Pasha et al. (2007)	300	Sodium bentonite	0-0.1	0-0.1	1-42
Pasha et al. (2008)	300	Sodium bentonite	0-1	0	1-42
Kermanshahi et al. (2009)	288	Sodium bentonite	0-1	0-0.5	1-42
Ghahri et al. (2010)	500	Sodium bentonite	0-0.5	0-0.5	1-35
Katouli et al. (2010)	448	Bentonite	0-3	0	1-42
Damiri et al. (2010)	288	Sodium bentonite	0-3	0	1-42
Manafi (2012)	336	Sodium bentonite	0-0.75	0-0.5	1-35
Damiri et al. (2012)	288	Sodium bentonite	0-3.75	0	1-42
Safaeikatouli et al. (2012)	448	Sodium bentonite	0-3	0	1-42
Indresh et al. (2013)	360	High-grade bentonite	0-0.75	0-0.5	1-35
Khanedar et al. (2013)	260	Sodium bentonite	0-1.5	0	1-42
Eckhardt et al. (2014)	1056	Ca Montmorillonite	0-0.5	0-3	1-42
Pappas et al. (2014)	300	Bentonite	0-1	0.02	1-42
Ani et al. (2014)	360	Bentonite	0-5	0	1-28
Dos Anjos et al. (2015)	250	Bentonite	0-0.75	0-2	1-21
Azizpour & Moghadam (2010)	300	Sodium bentonite	0-3	0-0.2	1-42
Malekinejad et al. (2015)	224	Bentonite	0-0.5	0-0.5	1-24

Table 1- Continued

Authors	*Birds (n)*	*Bentonite Form*	*Dosage (%)*	*Aflatoxin Level (mg/kg)*	*Period (day)*
Shannon et al. (2017)	180	Natural bentonite, Concentrated bentonite	0-0.5	0-2	1-21
Attar et al. (2017)	540	Sodium bentonite	0-1.5	0	25-42
Rafiu et al. (2019)	180	Sodium bentonite	0-0.15	Unknown	1-56
Basseboua et al. (2018)	420	Sodium bentonite	0-5	0	1-50
Abadi et al. (2019)	256	Sodium bentonite	0-3	0	1-42
Attar et al. (2019)	540	Sodium bentonite	0-1.5	0	11-42
Saleemi et al. (2020)	125	Bentonite	0-0.3	0-0.3	1-42
Mgbeahuruike et al. (2018)	80	Sodium bentonite	0-0.2	0-0.12	1-56

2.2. Data extraction and description

The variables used in the database were average daily gain (ADG), feed conversion ratio (FCR), daily feed intake (DFI), mortality, carcass yield, breast weight, leg weight, giblet weight (liver, heart, kidney, gizzard, pancreas, bursa of Fabricius), and blood constituents (albumin, total protein, uric acid, calcium, gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) glycogen, globulin, hemoglobin, packed cell value (PCV). Microbial population and intestinal shape were excluded in the database due to the small number of studies that provided the related parameter. All measured data were converted into the same units for statistical analysis and the dosage of bentonite was expressed in percentage (%) of diets. The data on broiler performances were divided into the starter phase, the final phase, and the cumulative. The database used in meta-analysis and descriptive statistics are presented in Table 1 and Table 2. The database was published between 2001 and 2020 with a total of 10 527 broiler chickens. Bentonite used were sodium bentonite, calcium bentonite, and montmorillonite form with levels ranging from 0 (control) to 5 %. Meanwhile, aflatoxin B1 levels in the diet ranged from 0 to 5 mg/kg. The minimum period of the evaluated study lasted 21 days, while the maximum period lasted 50 days. The broiler strains used were Cobb 500, Ross 308, Arbor Acres, Faobro, and Vencobb (Table 1).

Table 2- Regression equations for the effect of bentonite dose on broiler performances

Parameter	*Unit*	*n*	*Parameter estimates*					*Model statistics*		
			Model	*Intercept*	*SE intercept*	*Slope*	*SE slope*	*p-value*	*RMSE*	*AIC*
Starter phase										
ADG	g/bird/day	56	L	30.16	1.66	0.7115	0.554	<0.0001	9.642	351.0
DFI	g/bird/day	56	L	47.13	2.57	1.1235	0.703	<0.0001	12.114	380.5
FCR		56	L	1.57	0.06	0.001	0.009	<0.0001	0.156	-74.5
Mortality	%	10	L	3.83	2.66	-1.0158	4.021	0.3864	8.828	51.2
Finisher phase										
ADG	g/bird/day	70	L	59.6014	5.1638	1.2407	0.763	<0.0001	15.221	522.0
DFI	g/bird/day	68	L	111.67	10.2945	2.4961	1.265	<0.0001	25.243	596.0
FCR		70	L	2.0423	0.1008	-0.01552	0.014	<0.0001	0.280	-19.6
Cumulative										
ADG	g/bird/day	86	L	44.0454	1.9219	1.3514	0.666	<0.0001	13.859	601.2
DFI	g/bird/day	86	L	90.2034	4.4378	1.6860	1.145	<0.0001	23.489	700.9
FCR		86	L	2.1092	0.1104	-0.02356	0.0117	<0.0001	0.236	-40.2
Mortality	%	30	L	11.8190	3.4268	-1.1562	1.605	0.025	20.410	222.7

n: Number of treatments, RMSE: Root mean square error, SE: Standard error, AIC: Akaike information criterion, L: Linear

2.3. Statistical analysis

The data in the database were analyzed statistically in a meta-analysis using a mixed model methodology (St-Pierre 2001). The level of bentonites was fixed effects while the studies were random effects. The procedure for the mixed model was as follows:

Yij= B0+ B1Xij+ si+ eij

where Yij was the dependent variable, B0 was overall intercepted across all experiments (fixed effect), B1 was the linear regression coefficient of Y on X, Xij was the value of the continuous predictor variable (bentonite level), si was a random effect of experiment i, and eij was the unexplained residual error. The statistical analysis was conducted using SAS software version 9.1 and the p-value 0.05 criteria to assess the significant effect of each variable.

3. Results and Discussion

3.1. Performances

The regression equations between the level of bentonite and broiler performances are presented in Table 2. In general, our meta-analysis study shows that dietary bentonite supplementation indicates positive effects on broiler performances. ADG and DFI showed a linear increase ($p<0.001$) and FCR represented a linear decrease ($p<0.001$) in all phases. A higher bentonite level was correlated with an increase in ADG (Figure 1) with linear equation Y= 44.05+1.351X and a decrease in FCR with linear equation Y= 2.11–0.024X (Figure 2). The mortality rate linearly decreased ($p<0.05$) in cumulative by increasing bentonite level.

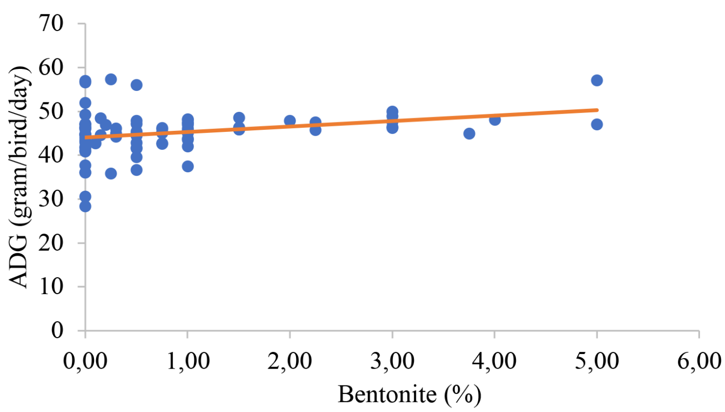

Figure 1- Relationship between bentonite levels and ADG cumulative based on the results of the meta-analysis. Equation: ADG cumulative (gram/bird/day) = 44.05+1.351×bentonite (%) (n=86; p<0.0001; RMSE=13.86)

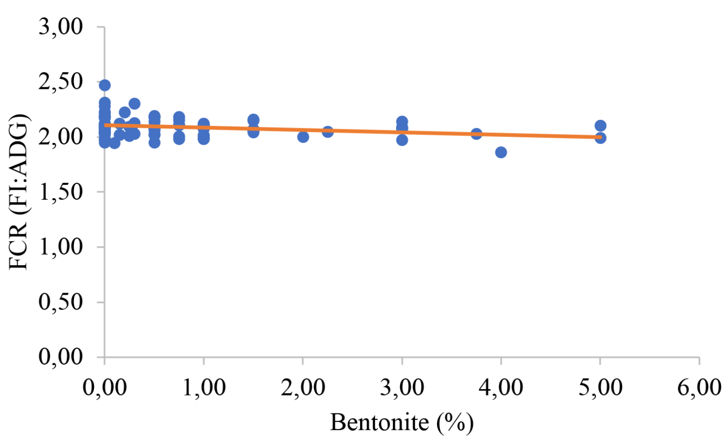

Figure 2- Relationship between bentonite levels and FCR cumulative (FI: ADG) based on the results of the meta-analysis. Equation: FCR cumulative =2.11–0.024 × bentonite (%) (n=86; p<0.0001; RMSE=0.24)

The improvement in ADG, DFI, and feed efficiency indicates that adding bentonite to the diet improves nutrient utilization and also suggests that bentonite has a beneficial effect on chickens fed an aflatoxin-contaminated diet. This result was supported by previous studies that used calcium bentonite and sodium bentonite (Khanedar et al. 2013; Besseboua et al. 2018). The positive impact of dietary bentonite could be attributed to the effect of bentonite in improving the digesta retention time, water absorption, and digestive enzymes in the intestine (Damiri et al. 2012; Khalifeh et al. 2012), nutrient digestibility (Pasha et al. 2008) and lowering in total counts of pathogenic bacteria (Shehata & Abd El-Shafi 2011) hence improving broiler performance. Moreover, bentonite improved intestine health and nutrient absorption by increasing villus height and surface area of broilers and enhancing regeneration of the intestinal epithelium (Wu et al. 2013; Besseboua et al. 2018).

Additionally, the efficacy of bentonite is related to its high mineral content including macro minerals (calcium, sodium, magnesium) and micro minerals (iodine, selenium, iron, zinc) which are needed to improve the enzymes activity and hormones to support growth (Smith et al. 2018). The presence of silicate minerals activity, which enhanced the transit time of feed in digestive tract and nutrient metabolism, might be responsible for performance improvement (Safaeikatouli et al. 2012). Suzanne et al. (2017) stated that clay groups have anionic microstructures in which most of the alkali metal ions and trace elements can be used as sources of animal minerals.

Another mechanism of bentonite in increasing the performance of broilers was through its ability to absorb aflatoxins B1 (Eckhardt et al. 2014; Mgbeahuruike et al. 2018). The previous study revealed that high doses of aflatoxin lead to acute clinical symptoms, increase liver damage, and decrease immune system. In broilers, aflatoxin was able to eliminate several enzymes activity involved in protein, starch, and lipids digestion, resulting in lower body weight increase and feed efficiency (Dos Anjos et al. 2015). The mechanisms of mycotoxins binding by bentonite had been widely investigated including ion interactions, electron donors, selective chemisorption, hydrogen and furan rings bonds as well as the interaction between carbonyl groups and exchange cations (Wang et al. 2018; Deng et al. 2010).

3.2. Percentage of carcass yield and giblet weight

The regression equations between the level of bentonite, carcass yield, and giblet percentage are presented in Table 3. The positive effects of bentonite addition in diet with a linear pattern ($p<0.001$) resulted in increasing carcass weight percentage, breast weight percentage, leg, bursa of Fabricius, and gizzard. Meanwhile, the percentage of kidney, liver, heart, and pancreas linearly decreased ($p<0.001$) by increasing bentonite level. The increase in the carcass yield revealed the efficacy of bentonite in improving digestive enzymes and nutrient digestibility in the intestine as well as protecting the giblet against aflatoxins, as result, it was able to work normally during digestion, absorption, and metabolism. The efficacy of bentonite may also be recognized in the reduction of liver and kidney size that is susceptible to aflatoxins. This argument is confirmed by Dos Anjos et al. (2015) who claimed that the inclusion of bentonite to aflatoxin B1 diet reduced the prevalence of histological lesions, the proportion of kidney and liver weights of chicks. Aflatoxins are recognized as irritating to the digestive tract and causing intestinal dysfunction, increasing the relative weights of giblets, including liver, kidneys, heart, bursa fabricius, and pancreas (Valchev et al. 2013). Additionally, histological results revealed hepatocellular degeneration lesions in the livers of aflatoxin-exposed birds, as well as vacuolar degeneration and significant fat accumulation caused by decreased lipid transport rather than enhanced lipid biosynthesis (Cruz et al. 2019). In birds, the kidney is involved in the clearance of hazardous metabolic waste products from the blood as well as the preservation of biochemical homeostasis. Because of these activities, the kidney is the primary organ in poultry that accumulates Aflatoxin B1. Several studies have found that Aflatoxin B1 induces renal dysfunction, which might explain the lower 1, 25-dihydroxycalciferol level, calcium, and phosphorus (Gholami-Ahangaran et al. 2016; Fouad et al. 2019). This statement could also explain why the calcium content of the blood increased with bentonite inclusion in this study.

Table 3- Regression equations for the effect of bentonite dose on giblet and carcass yield

Parameter	*Unit*	*n*	*Parameter estimates*					*Model statistics*		
			Model	*Intercept*	*SE intercept*	*Slope*	*SE slope*	*p-value*	*RMSE*	*AIC*
Carcass	%	24	L	60.94	4.24	0.594	0.747	<0.0001	3.376	115.1
Breast	%	22	L	21.81	2.91	0.036	0.294	<0.0001	2.162	84.7
Leg	%	22	L	23.81	3.35	0.116	0.269	<0.0001	1.972	82.4
Bursa of Fabricius	%	34	L	0.15	0.04	0.008	0.009	<0.0001	0.051	-104.9
Gizzard	%	45	L	2.29	0.26	0.028	0.039	<0.0001	0.329	25.2
Heart	%	24	L	0.59	0.03	-0.009	0.026	0.0354	0.143	-34.2
Kidney	%	52	L	0.75	0.08	-0.098	0.098	<0.0001	0.446	17.2
Liver	%	80	L	3.36	0.35	-0.172	0.197	<0.0001	2.353	287.0
Pancreas	%	26	L	0.37	0.049	-0.014	0.013	<0.0001	0.063	-68.1

n: Number of treatments, RMSE: Root mean square error, SE: Standard error, AIC: Akaike information criterion, L: Linear

3.3. Blood constituents

The regression equations between the level of bentonite and metabolites parameters of broiler chickens are presented in Table 4. The increase of dietary bentonite increased total protein, albumin, hemoglobin, aspartate aminotransferase, calcium, glycogen, and globulin (linear pattern; $p<0.001$). There was a linear decrease ($p<0.001$) in uric acid and gamma-glutamyltransferase. Our study shows that the indication of bentonite inclusion in the broiler diet increases the absorption of aflatoxin in the digestive system, which inhibits the decrease of blood serum albumin, globulin, hemoglobin, PCV, and total protein when using a contaminated diet. In previous studies, the effect of bentonite inclusion in the broiler chicken diet increased albumin, total protein, and globulin in the serum (Aghashahi 2015; Shannon et al. 2017). The efficacy of bentonite may be attributed to its composition, which is mostly in the form of adsorption montmorillonite (Pouraboulghasem et al. 2016). Montmorillonite has the ability to inhibit uric acid absorption in the gut by increasing uric acid diffusion from the bloodstream to the intestine (Ma et al. 2009). The addition of bentonite to the aflatoxin diet was also beneficial in avoiding alterations in GGT enzyme concentrations that were highly correlated with the damage of the liver as the main target organ. As a result, the decrease in GGT suggested that bentonite alleviated the negative effects of aflatoxin (Shannon et al. 2017). The decrease in hemoglobin and PCV were markedly reduced in the broiler chicks fed containing aflatoxin reported by Rathod et al. (2017). The low proportion of PCV might be due to aflatoxin-induced hemolysis caused by plasma membrane lipid peroxidation (Umar et al. 2012). Meanwhile, the decrease in hemoglobin concentration during aflatoxicosis might be attributed to a combination of decreased iron absorption and hematopoiesis suppression (Abeena et al. 2015).

Table 4- Regression equations on the influence of bentonite dosage blood parameter

Parameter	*Unit*	*n*	*Parameter estimates*					*Model statistics*		
			Model	*Intercept*	*SE intercept*	*Slope*	*SE slope*	*p-value*	*RMSE*	*AIC*
Albumin	g/dL	51	L	1.32	0.17	0.119	0.106	<0.0001	0.570	49.2
Total Protein	g/dL	51	L	2.74	0.29	0.116	0.185	<0.0001	1.001	103.2
Calcium	mg/dL	15	L	9.92	0.80	0.13	0.09	<0.0001	0.54	19.0
Uric acid	mg/dL	25	L	5.77	0.66	-0.414	0.414	0.0001	2.218	85.1
AST	U/L	15	L	39.96	6.57	1.210	1.281	<0.0001	6.104	85.4
GGT	U/L	21	L	11.49	2.42	-1.891	2.288	0.0009	5.819	109.5
Glycogen	mg/dL	14	L	224.16	17.95	24.846	20.904	<0.0001	56.695	119.2
Globulin	g/dL	22	L	1.69	0.41	0.069	0.144	<0.0001	0.449	18.6
Hemoglobin	g/dL	12	L	9.60	1.34	1.132	2.428	0.0141	2.694	48.0
PCV	%	12	L	34.36	3.74	11.300	13.019	0.2879	14.559	77.5

n: Number of treatments, RMSE: Root mean square error, SE: Standard error; AIC: Akaike information criterion, L: Linear

Generally, by meta-analysis, the use of bentonite benefited broiler performance in an aflatoxin-contaminated diet. Nonetheless, it should be recognized that bentonite is also able to absorb the nutritional content in animal diets including micronutrients as well as veterinary drugs (Alam & Deng 2017). Because bentonite has no specific effects on mycotoxin binders, The European Food Safety Authority (EFSA) has advised that oral veterinary drugs be evaluated in the diet containing a mycotoxin binder to ensure their safety in terms of binding to the drug components. Therefore, EFSA recommended using no more than 20 g/kg of bentonite in the diet with the composition of smectite (>70%), opal and feldspar (<10%), and calcite and quartz (4%) for effectiveness in binding aflatoxin B1 and safe for all animals (EFSA 2012).

4. Conclusion

The bentonite addition has the ability to eliminate the detrimental effects of aflatoxin which may increase the broiler performance (ADG, DFI, FCR, and mortality rate). Bentonite is also effective in improving carcass yield and preventing changes in abnormal giblet weights caused by aflatoxin. However, for effectiveness and animal safety, the EFSA recommends using a maximum of 2% bentonite in the feed.

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Authorship Contributions: Concept: A.D., A.J., E.O., Design: A.D., A.J., E.O., Data Collection or Processing: A.D., Analysis or Interpretation: A.D., A.J., Literature Search: A.D., Writing: A.D., A.J., E.O.

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