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RESEARCH ARTICLE

Investigations into *Salmonella* contamination in feed production chain in Karst rural areas of China

Shenglin Yang[1] **· Zongfen Wu**[1,2] **· Wei Lin**[1] **· Longxin Xu**[3] **· Long Cheng**[4] **· Lin Zhou**[1]

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Abstract In order to understand the status of *Salmonella* contamination of feed production chain in Karst rural areas, southwest of China, a total of 1077 feed samples including animal feed materials and feed products were randomly collected from different sectors of feed chain covering feed mills, farms, and feed sales in nine regions of Karst rural areas between 2009 and 2012, to conduct *Salmonella* test. The different positive rates with *Salmonella* contamination were detected, the highest was 4.7 % in 2009, the lowest was 0.66 % in 2011, while 4.3 % in 2010, 2.8 % in 2012, respectively. Twelve types of feed including concentrate, complete, self-made, and feed ingredients were inspected. *Salmonella* contamination mainly concentrated on animal protein material such as meat meal, meat and bone meal, feather meal, blood meal, and fish meal. No *Salmonella* contamination was detected in feed yeast, microbial protein, rapeseed, and soybean meal. *Salmonella* contamination existed in each sector of feed production chain. This investigation provided a basic reference for feed production management and quality control in feed production chain in Karst rural areas of China.

Keywords *Salmonella* · Contamination · Feed · Ingredient

Introduction

Animal feeds contaminated with *Salmonella* pose a risk of infection to livestock and thus to the human food chain (Crump et al. 2002; EFSA 2008). In humans, it is considered one of the most frequent bacterial foodborne diseases posing major public health threats in industrialized countries (D'Aoust 1997). In the USA, it has been estimated that 1.4 million people are infected with nontyphoidal *Salmonella*, resulting in 15,000 hospitalizations and approximately 400 deaths every year (Voetsch et al. 2004). Approximately 6000 to 12,000 cases of *Salmonellosis* are reported annually in Canada (http://dsp-psd.pwgsc.gc.ca/collection/HP3-1-32-20.p). There were about 152,000 confirmed cases of human salmonellosis were reported within the EU in 2007 (EFSA 2009), but the decreasing trend in confirmed salmonellosis cases was reported from 2008 to 2012. A total of 92,916

Responsible editor: Philippe Garrigues

✉ Shenglin Yang
shenglinyang@126.com

Zongfen Wu
wzf69@foxmail.com

Wei Lin
229297498@qq.com

Longxin Xu
shangaorenyegao@163.com

Long Cheng
Paul.cheng@lincoln.ac.nz

Lin Zhou
916841843@qq.com

[1] College of Animal Science, Guizhou University, Guiyang, Guizhou Province 550025, China

[2] Monitoring Institute of Feed and Veterinary Drug of Guizhou Province, Guiyang, Guizhou 550005, China

[3] Institute of Guizhou Husbandry and Veterinary, Guiyang, Guizhou 550000, China

[4] Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 84, Lincoln, New Zealand

salmonellosis cases were reported by the 27 European Union Member States, with 91,034 confirmed cases in 2012 (EFSA 2014). As a zoonotic agent, animals as well as wildlife can be important contributors to the abundance and distribution of *Salmonella* in the environment and possible transmission to humans (Smith et al. 2005; Ashbolt and Kirk 2006; Compton et al. 2008; Handeland et al. 2008; Hernandez et al. 2012). It has been estimated that more than 34,500 symptomatic infections with *Salmonella* spp. occur annually in the Netherlands (about 16.5 million population) (Havelaar et al. 2009). With Enteritidis and Typhimurium being the two most frequently isolated *Salmonella* enterica subsp. enterica serotypes, accounting for 29 % (Enteritidis) and 47 % (Typhimurium, including its monophasic variant 4) (van den Brandhof et al. 2004). *Salmonella* contamination in human mostly related to animal product through animal ingestion of *Salmonella* contaminated feed (Davies and Wales 2010). Studies have shown strong links between *Salmonella* contamination of feedstuffs or feed mills and infections, with *Salmonellas* of the same serovars, of groups of chickens (Davies et al. 2001; Jinghui et al. 2014), turkeys (Zecha et al. 1977; Nayak et al. 2003), pigs (Newell et al. 1959, Joost et al. 2013), goats (Duffy et al. 2009), cattle (Glickman et al. 1981; Min et al. 2014), small ruminants, and wild-living birds (Pao et al. 2014).

The importance of animal feed as source for *Salmonella* dissemination has been highlighted by EFSA (European Food Safety Authority), particularly in countries with a low prevalence of *Salmonella* in primary production (EFSA 2008). Animal feedstuffs are at risk of *Salmonella* contamination at several stages in the feed chain starting with the production of ingredients. Different factors are known to influence the risk of introducing *Salmonella* into animal feed, including contaminated ingredients, contaminated feed mill environments causing re-infection of the feed, wild birds, and rodents (Hoszowski et al. 2008; Andrés-Barranco et al. 2016). According to European feed legislation, Regulation (EC) No. 183/2005, feed business operators should apply Hazard Analysis and Critical Control Points (HACCP) principles and good hygiene practice/good manufacturing procedures (GHP/GMP) at each stage of the feed chain in order to secure safe feed (EFSA 2014). At present, China has taken strict detection and control measurements of Salmonella animal feed. Animal feed could not be contaminated with *Salmonella* according to Chinese official regulation GB13078-2001 (SAPRC. 2001). It has been reported that the feed yield in China in 2011 was 200 million tons, ranged the first in the world and surpassed firstly United States, feed mill almost distributed the whole country (CFIA 2013). However, the situation of animal feed contaminated with *Salmonella* in China was relatively serious compared to the USA, Japan, and some European countries. It was found that non-typhoid *Salmonella* annually caused 9.874 million gastroenteritis cases in China and 91.5 % of them were caused by food transmission (Mao et al. 2011). Several reports have shown animal feed and food contamination with *Salmonella* existed in different provinces such as Henan, Sichuan, Guangdong, Fujian, Xinjiang, and Gansu (Chunhong et al. 2004; Qiong et al. 2006; Yuanran et al. 2013). In these reports, the degree of contamination with *Salmonella* in raw materials, especially in animal proteinaceous feed materials, was more serious than that in compound feeds. Jijun and Biao (2009) reported the positive rate of 18.2 % of fish meal and 15.4 % of meat and bone meal contaminated with *Salmonella* from 299 samples, while 10.1 % positive rate for the finished feeds and 7.8 % positive rate for the concentrated feeds. Additionally, *Salmonella* contamination could be observed based on the above studies in different sections of food chain.

The Karst rural area, an undeveloped district located in the southern west of China, has been recently listed as an ecological agricultural developing region by the Chinese government due to its specific geographical and ecological environment. There are more than 200 enterprises for feed production and sales. However, there appears to be very little literature concerning feed production and food chains with *Salmonella* contamination in this area. To clarify the mechanisms of the contamination, accumulation of basic data is necessary for local government to draw up the plan to control *Salmonella* contamination in feed production chain. In the present study, samples were collected from the different sections in feed production chain in Karst rural areas of China from the year 2009 to 2012, and the data used for analysis in this research can be as a reference to feed production in the other areas of China. It provides a basic data to evaluate *Salmonella* contamination in feed production chain and helps to identify the contamination sources and sites. Therefore, the feed quality supervision system may be improved accordingly.

Materials and methods

Sample collection

All samples used for this study were collected from different departments distributed in nine regions of Karst rural areas in China between 2009 and 2012. Sampling sites included feed mills, feedstuff shops, and farms, where represented the feed production chain in whole Karst areas. The total of 1077 samples were collected, 234 of which were taken in 2009, 328 in 2010, 301 in 2011, and 214 in Table 1. Sampling collecting method meet the standard of GB/T14699.1 in China. For each kind of sample, three packs of the same samples (500 g for each) with a total of 1500 g of weight were collected, which could meet the requirements of examination for three laboratories. For the test of *Salmonella*, 8 analytical samples of 25 g were tested.

Table 1 Number of samples collected from nine regions from 2009 to 2012 in Karst rural areas

Areas	2009	2010	2011	2012	Total
Guiyang	64	127	88	65	344
Zunyi	42	45	43	43	173
Ansun	32	41	41	28	142
Qiannan	14	23	14	14	65
Qiandongnan	12	16	33	10	71
Tongren	24	14	14	14	66
Biye	15	23	34	18	90
Liupanshui	21	12	16	11	60
Qianxinan	10	17	18	11	66
Total	234	328	301	214	1077

Reagent and broth preparation

Biochemical reagents including buffered peptone water (BPW), enrichment broth with magnesium chloride, enrichment broth with cystine, and selenite were purchased from China import and export commodity inspection institute; Phenol red agar, deoxycholate hyadrogen sulfiale lactose agar (DHL), magnesium chloride malachite green medium, nutritive agar were from Hangzhou microbial Co. Ltd. China. API ID32 reagent strips were made up of 32 small dry medium tubes, 3 ml ampoule of 0.85 % NaCl solution, JAMES reagent, petroleum wax were produced by French Merieux biological company, purchased from Guangzhou QianJiang Company, China. A–F polyvalent serum was from Lanzhou biological products research institute, the ministry of health of China.

Bacteriological examination

The detection of *Salmonella* from feed was based on culture methods according to the national standard of the People's Republic of China (GB - T13091-2002) (19). Briefly, *Salmonella* was cultured on phenol red agar, the plates were incubated at 37 °C for 24 h, and the suspicious bacteria was identified in DHL plate when colors of cell edges or whole cell appeared black. Then, the suspicious bacterial colonies were picked up for culturing on nutrient agar plate at 37 °C for 18–24 h. The pure cultures were taken out for biochemical test using the API bacteria identification system and results were read automatically on ATB Express/mini API (BIOMÉRIEUX, France); strains suspected of being *Salmonella* were used to conduct further serological identification.

Biochemical identification of *Salmonella* bacteria

Twenty-five grams of sample each was emulsified and then inoculated into the 500-ml flask with 225-ml BPW buffering peptone, incubated at 37 °C for 8 to 18 h. Ten milliliters of enrichment broth cultures were inoculated into 100-ml selenite cystine medium and incubated at 37 °C for 24 h. At the same time, 10-ml broth cultures were inoculated into 100 ml of magnesium chloride malachite green medium at 42 °C for 24 h. Two sorts of enrichment broth were separately cultured and inoculated on DHL and phenol red agar plates, then incubated at 37 °C for 20–24 h.

Serological test

The suspected small bacteria colonies growing on phenol red agar whose medium color changing from pink to red were selected to conduct the further biochemical identification using the API automatic verification system with ID32 reagent strips. *Salmonella* isolates were obtained and identified to serovar level according to the Kauffmann-White scheme (Grimont and Weill 2007).

Results

Contamination of various kinds of feeds with *Salmonella*

In total, 1077 samples were collected and analyzed from nine regions of Guizhou province in China from 2009 to 2012 during this study (Table 1). In 2009, a total of 234 samples were determined, 42 of which represented 8 sorts of feed materials, 106 of which were commercial finished feed samples, 85 of which were commercial concentrated feed samples, and 1 of which was the sample of self-made by farm. The results showed that among all samples, 11 of which were contaminated with *Salmonella*, and the *Salmonella* contamination rate were 4.7 %. The high positive rates were detected in meat meal, meat and bone meal, feather meal, blood meal, and fish meal. No *Salmonella* contamination was found in commercial finished feeds, commercial concentrated feeds, bone meal, and feeding yeast (Table 2). In 2010, among 328 samples, 11 of which were contaminated with *Salmonella* (4.3 %). In 2011, only 2 samples were positive among 301 samples (0.66 %), while 6 samples contaminated among 214 samples (2.8 %) in 2012. Compared to the positive rates of contaminated with *Salmonella* from 2009 to 2012, *Salmonella* contamination mainly distributed in animal proteinaceous materials such as meat meal, meat and bone meal, feather meal, blood meal, and fish meal. No *Salmonella* contamination was observed in vegetable protein material like rapeseed or soybean meal, but some *Salmonella* contamination was found in the concentrated feed and the self-made feed by farm (Fig. 1).

Table 2 Number of samples contaminated with *Salmonella* from different types of feeds from 2009 to 2012

Category	2009			2010			2011			2012		
	No. examined samples	No. positive samples	Positive rate (%)	No. examined samples	No. positive samples	Positive rate (%)	No. examined samples	No. positive samples	Positive rate (%)	No. examined samples	No. positive samples	Positive rate (%)
Concentrated feed	85	0	0	167	5	3	143	1	0.7	143	4	2.8
Finished feed	106	0	0	104	0	0	131	0	0	50	0	0
Self-made by farm	1	1	100	8	0	0	0	0	0	0	0	0
Meat and bone meal	7	1	14.3	26	6	23	6	0	0	7	2	28.6
Bone meal	2	0	0	3	1	33.3	0	0	0	0	0	0
Meat meal	10	5	50	0	0	0	4	0	0	0	0	0
Feather meal	4	1	25	2	1	50	0	0	0	0	0	0
Blood meal	5	1	20	3	0	0	4	1	25	0	0	0
Fish meal (domestic)	6	1	16.7	8	1	12.5	8	0	0	2	0	0
Fish meal (imported)	5	1	20	2	0	0	4	0	0	5	0	0
Feeding yeast	3	0	0	3	0	0	0	0	0	0	0	0
Microprotein	0	0	0	2	0	0	1	0	0	0	0	0
Rapeseed or soybean meal	0	0	0	0	0	0	0	0	0	7	0	0
Total	234	11	4.7	328	14	4.3	301	2	0.66	214	6	2.80

Concentrated feed means a special feed product mixed with vegetarian and animal proteinaceous materials, vitamines, minerals, e.g., but no energic materials; finished feed means a kind of compound feedstuff with complete nutritive values; self-made by farm means a compound feedstuff made by farm itself can be fed to animals directly

Contamination of production sections with *Salmonella* in feed chain

Salmonella contamination status from different sections of feed production chain was also investigated from 2009 to 2012. Table 3 shows a total 1077 samples inspected, 33 sample appeared to be positive (3.1 %), 4 of them were from feed ingredients, 5 of which were from feedstuff in feed mill, 7 were from sale sections, 13 of them were from processing of feed ingredients, 4 from feedstuff in farms. These results imply that there was *Salmonella* contamination existed in each section of feed chain. In 33 positive samples, the animal proteinaceous materials with *Salmonella* contamination reached 63.6 %, the concentrated feeds were 30.3 %, self-made feed by farm was only 3.0 %. No positive samples were detected in vegetable proteinaceous materials. As to sections of feed production chain, section of feed materials processing had the highest proportion *Salmonella* contamination (39.4 %), followed by section of compound feed in sales (21.2 %). Section of compound feed in feed mill, section of feed materials in feed mill, and section of compound feed in farm were 15.2, 12.1, and 12.1 %, respectively.

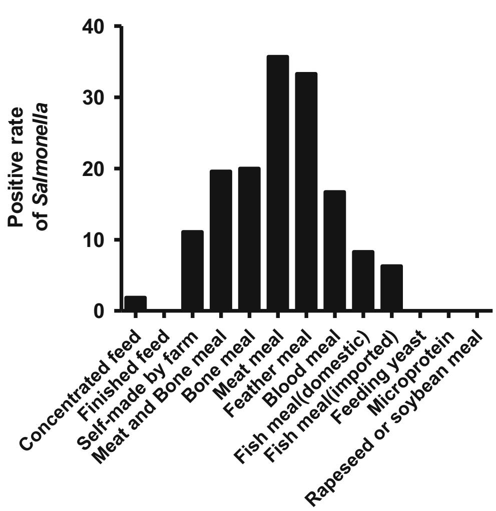

Fig. 1 Positive rate of *Salmonella* contamination from feedstuff and feed ingredients in Karst rural areas of China during 2009–2012

Effects of processing methods on feed contaminated with *Salmonella*

Effects of two kinds of processing methods on feed contaminated with *Salmonella* are listed in Table 4; the results indicated that no *Salmonella* contamination was detected in 775 samples collected from the feed processed with bulking method, while 21 samples were found to be positive from 35 feed samples processed with pelletizing method. The temperature for bulking ranged from 93.3 to 178.6 °C for 45–60 s, moisture content of puffing feed was 25–35 %, while the temperature for pelletizing was 71.1 to 82.2 °C for 2–16 s, moisture content of pelleting feed was 11–19 %, the temperature for bulking was significantly higher than that for pelletizing, the time spent for puffing also more than that for pelletizing. This may explain why no *Salmonella* contamination rate was detected for bulking method.

Discussion

There was an overall reduction tendency in positive rate of *Salmonella* contamination. It reflects that the feed producers gradually improved the consciousness of avoiding *Salmonella*

Table 3 *Salmonella* contamination in different sections of feed production chain

Category	No.examined samples	No. positive samples	Positive rate (%)	Feed materials in feed mill	Compound feed in feed mill	Compound feed in sales	Feed materials in processing	Compound feed in farms
Concentrated feed	538	10	1.86	0	5	5	0	0
Finished feed	391	0	0	0	0	0	0	0
Self-made by farm	9	1	11.1	0	0	0	0	1
Meat and bone meal	46	9	19.6	2	0	1	6	0
Bone meal	5	1	20	0	0	0	0	1
Meat meal	14	5	35.7	0	0	0	5	0
Feather meal	6	2	33.3	0	0	0	2	0
Blood meal	12	2	16.7	0	0	0	0	2
Fish meal (domestic)	24	2	8.3	2	0	0	0	0
Fish meal (imported)	16	1	6.3	0	0	1	0	0
Feeding yeast	3	0	0	0	0	0	0	0
Microprotein	6	0	0	0	0	0	0	0
Rapeseed or soybean meal	7	0	0	0	0	0	0	0
Total	1077	33	3.1	4.0	5.0	7	13	4.0

Concentrated feed means a special feed product mixed with vegetarian and animal proteinaceous materials, vitamines, minerals, etc. but no energic materials; finished feed means a kind of compound feedstuff with complete nutritive values; self-made by farm means a compound feedstuff made by farm itself can be fed to animals directly

contamination in feed production. Although there were differences in sampling numbers and sampling types for examination from 2009 to 2012, the sources of *Salmonella* contamination mainly came from animal feed raw material especially from meat and bone meal and blood meal. Vegetable protein materials such as rapeseed meal and soybean meal had not been found positive. This result is consistent with the previous reports (Hacking et al. 1978; Nabbut 1978; Harris et al. 1997). The reason is twofold: (1) the vegetable fat has been shown to inhibit the growth of Gram negative bacteria (Khan and Atamy 1969; Fay and Farias 1975) and (2) high temperatures (up to 105 °C) may eliminate *Salmonella* when rapeseed meal and soybean meal being processed (Wierup and Kristoffersen 2014). However, it has been reported that *Salmonella* was frequently isolated from consignments of vegetable proteins as feed materials (Martin and Per 2010; Wierup and Häggblom 2010; Wierup and Kristoffersen 2014). In a Dutch report, 3.2 and 6.7 % of Brazilian extracted soybeans were found positive for *Salmonella* during 2002 and 2003, respectively (Anon 2003). In a recent comprehensive study based on an annual examination of up to 80,000 lots of feed, Kwiatek et al. (2008) reported that in Poland up to 15.0 and 15.4 % of imported lots of soy and rapeseed were respectively

Table 4 Comparison of *Salmonella* contamination of two processing methods

	Bulking	Pelletizing
No. examined samples	775	35
Temperature (°C)	93.3–176.8	71.1–82.2
Time (S)	45–60	2–16
Moisture content (%)	25–35	11–19
No. positive samples	0	21(60 % positive rate)

Salmonella contaminated in 2005–2007. From 1994 to 2012 in a Norwegian, 34 % of samples collected during unloading of ships delivering soybeans yielded *Salmonella*; the proportion of samples from ships that yielded *Salmonella* varied from 12 to 62 % each year. Corresponding data for products produced in Poland were 6.3 and 7.7 %. In the present study, the rapeseed meal was from the southwestern provinces, while soybean meal came from northeastern China. Heat treatment is the mandatory procedure in producing of materials. Steam (water vapor) is used during process of pelleted feedstuff, and it can be used for sterilizing of feed material as well. Although the situation, to some extent, seems to be safety, the potential risks still exist for feed producers when imported vegetable proteinaceous materials from other provinces or the other countries because oil seed feed ingredients are often contaminated by *Salmonella*. Besides, it is difficult to compare the level of contamination between different studies because the results depend on the sampling and culture techniques applied (Martin and Per 2010). Therefore, it is essential for feed producers in Karst rural areas to introduce HACCP-control to reduce the risk of *Salmonella* contamination. Heat treatment can also prevent *Salmonella* contamination of feed ingredients to be compounded feed.

Concentrated feed is also important source of *Salmonella* contamination. It may be caused by the difference feed processing methods: no heat treatment process in production of concentrated feed, only a mixture of proteinaceous materials and the multiple kinds of vitamin and minerals. In China, concentrated feed has a large market space because of its convenience, particularly in Karst rural areas, small farms can make full use of agricultural byproducts to reduce feed costs through mixturing concentrated feed. It is not necessary for small farms to invest money to establish feed mill. However, attentions must be paid in *Salmonella* contamination when using concentrated feed because of its high levels of proteins materials. In addition, the humid air especially in Karst rural areas easily leads to moldy feed. For large farms, pelleting and puffing feeds are often used because they have their own feed mills. Both pelleting and puffing need a heat treatment in feed processing. Pelleting usually involves temperatures between 70 and 90 °C (though pelleting at 90 °C may result in “slushy” feed) and may destroy 99 % of total bacteria (Furuta et al. 1980; Mccapes et al. 1989). In the EU, the control and elimination of *Salmonella* contamination in the crushing plants are important ways to prevent *Salmonella* introduction into EU farms. Control of *Salmonella* in crushing plants is feasible if recontamination is prevented since the production process reaches temperatures (up to 105 °C) that would eliminate *Salmonella* (Wierup and Kristoffersen 2014). This suggests that physical or chemical approaches are of importance for the raw materials and concentrated feed to eliminate *Salmonella* contamination prior to mixture of ingredients. A large number of reports demonstrated that *Salmonella* contamination could be reduced by chemical amendments, heat treatment, and irradiation (Leeson and Marcotte 1993; Jaquette et al. 1996). Heat treatments may also be more effective when used in conjunction with chemical treatments such as propionic acid, even though the effect may not be synergistic (Mattlho et al. 1997). Cold plasma is a relatively new process being developed for applications to fresh and fresh-cut fruits, vegetables, and nuts (Niemira and Gutsol 2010). Brendan (2012) reported that short treatments with cold plasma significantly reduced the viability of *E. coli* O157:H7 and *Salmonella* on the surfaces of almonds.

In our study, samples taken from all sections of feed production chain had been inspected to be positive for *Salmonella*, particularly in sections of feed materials processing, and the positive rate of *Salmonella* contamination was relatively high (39.4 %). The consequence is in line with other investigations (Chunhong et al. 2004; Qiong et al. 2006; Yuanran et al. 2013). It is worth mentioning that feed inspection is not only an effective way to check *Salmonella* contamination. It has been verified that HACCP associated with *Salmonella* contamination control is the best way (Martin and Per 2010). In China, there are entirely differences between manufacturing of finished feed and processing of feed materials in different provinces, and no consistent criterion can be provided for preventing *Salmonella* contamination. Until now, the feed quality tracing system for *Salmonella* contamination has not been established. This can attributed to the following aspects: firstly, although most of feed mills possess the basic conditions for inspection of *Salmonella* contamination, the consciousness of feed quality assurance is not strong. Most of mini-manufacturers of feed materials in Karst rural areas have poor conditions in professional people and facilities for *Salmonella* inspection. Secondly, the level to control feed quality does not meet the requirements of national standards, causing some unqualified animal raw materials were used. Thirdly, potential safety risk exists in sections of transportation and sales in feed chain and lack of related control measures. Fourthly, feed mills were not able to invest enough money to improve inspection technology and facilities. Finally, most of animal farms have their own rooms for feed processing with a simple grinder and mixer; they used to use blended mash without heat treatment.

Considerable researches reveal that heat treatment involving cooking and pelleting may destroy 99 % of total bacteria (Halls and Tallentire 1978). Bulking feed is an important type for piglets and chicks due to its high digestive ability and hygiene. In this study, two kinds of processing methods with different temperatures and heating time were compared and result showed a higher effectiveness in bulking procedure. There was a higher temperature (93.3–176.8 °C), a longer heating time (45–60 s), and a higher moisture content (25–35 %) in bulking procedure than that in pelleting procedure. This might be a reason why no positive rate detected in bulking procedure while 60 % positive rate detected in pelleting procedure. This result is in consistent with the report by Himathonkham et al. (1996). Additionally, the other method such as irradiation has been investigated as the alternative feed treatment. When vegetative bacteria are exposed to gamma rays, the structure of bacterial DNA is changed, resulting in a reduction in bacterial populations (Clavero et al. 1994; Aude et al. 2013). This method, here, has not been designed to conduct a trial in this study but it may be considered as a selective measure to reduce feed contaminated with *Salmonella.*

Conclusion

Feed contaminated with *Salmonella* in Karst rural areas, the southwest of China, mainly comes from animal feed ingredients, particularly from animal proteinaceous materials such as meat meal, meat and bone meal, feather meal, and blood meal. Most of the sections of feed chain have been monitored to be positive for *Salmonella* in these areas.

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