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Review Article

Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review

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ABSTRACT

Previously, fermentation has been associated with methods that improve the nutritional value of unconventional feed ingredients for broilers. In recent decades, the fermentation process has been employed to produce functional feeds that have the potential to improve broiler gastrointestinal tract microecology, health and production performance. Some of the functional ingredients found in fermented feed include lactic acid bacteria (LAB), lactic acid and other organic acids, and appear to play major roles in determining the beneficial effects of fermented feed on broiler gut health and performance. Unlike the pig, the available literature on broiler fermented feed is still rather limited. This review describes recent advances in the use of fermented feed (on the basis of conventional and unconventional feed ingredients) in broilers. Similarly, this review also shows that additional research is necessary to explore fermented feed as a viable food source in broiler nutrition.

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1. Introduction

In commercial poultry, the production of broiler feed contributes up to 70% of the total production cost. Due to increases in global feed prices, there is now a tendency in the poultry industry to move towards alternative or unconventional feed ingredients. This move is however limited by several issues: high and low fibre and protein contents and the presences of antinutritional factors (ANF) in unconventional feed ingredients that can reduce feed digestibility. Previous studies have shown that fermentation increased crude protein content but decreased crude fibre content (Khempaka et al., 2014; Sugiharto et al., 2015a, 2016a), several ANF

and toxic compounds in feed ingredients (Chiang et al., 2010; Xu et al., 2012).

Apart from improved nutritional properties, fermentation is associated with a high number of lactic acid bacteria (LAB), a low pH and a high concentration of organic acids (Engberg et al., 2009; Canibe and Jensen, 2012). It has been shown that these latter features alone or in combination, may protect the feed from pathogen contamination prior to feeding (Niba et al., 2009), benefit chicken gastrointestinal health (Missotten et al., 2013; Sun et al., 2013a) and chicken growth and development (Xie et al., 2016). Feeding fermented products has been commonly practiced in the pig nutrition arena for many years (Canibe and Jensen, 2012), however there is now increasing interest in incorporating fermented feed into broiler rations to take advantages of its positive influences, particularly on gut health and production parameters (Alshelmani et al., 2016; Zhang et al., 2016). In terms of cost efficiencies, the replacement of expensive conventional feedstuffs such as yellow corn in broiler diets may further encourage the use of cheaper unconventional fermented feedstuffs in broiler nutrition (Supriyati et al., 2015; Sugiharto et al., 2016a,b). This review gathers the current state of the art in chicken broiler nutrition and describes how the application of fermented feed in chicken broiler diets could

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influences growth performance, gastrointestinal tract microecology and immune responses.

2. Fermentation methods

Fermentation is a dynamic process involving microorganisms, substrates and environmental conditions to convert complex substrates into simpler compounds (Niba et al., 2009). It has been reported that fermentation outcomes can be highly variable, and appear to depend on the nature and characteristic of the substrates used (Canibe and Jensen, 2012; Subramanian and Vimala, 2012). Conditions including temperature, pH, the nature and composition of the medium, dissolved O₂ and CO₂, operational systems (e.g., batch, fed-batch, continuous), addition of precursors, mixing (cycling through varying environments) and fermenter shear rates, and the length of the fermentation process may all influence the rate of fermentation and quality of the fermented products (Renge et al., 2012). Depending on the type of microorganisms involved, fermentation will result in the formation of different final products such as lactic acid, ethanol or acetic acid, as different microorganisms may react differently to each substrate (Niba et al., 2009; Subramanian and Vimala, 2012), e.g. *Lactobacillus* produce lactic acid, mould yield citric acid, whereas yeasts generate ethanol and CO₂ (Couto and Sanroman, 2006).

Broadly, there are 2 types of fermentation techniques, i.e., solid state fermentation (SSF) and submerged fermentation (SmF) (Couto and Sanroman, 2006; Subramanian and Vimala, 2012). The SSF approach involves solid substrates such as grains, rice, rice bran and wheat bran in the absence of free-flowing liquid (Couto and Sanroman, 2006; Supriyati et al., 2015). Thus, SSF is generally exploited for the production of fermented dry feed (FDF) which can be added to basic feed mixes such as whole grain, or the FDF can be crimped or produced in powder form. Due to the low moisture content, the SSF method can only be carried out by a limited number of microorganisms, mainly the fungi such as *Aspergillus* spp. and *Rhizopus* spp., although some bacteria like *Lactobacillus* spp. can also be used (Couto and Sanroman, 2006; Supriyati et al., 2015).

Unlike the SSF method, the SmF approach requires free-flowing liquid substrates such as broth media, molasses, whey and wet distillers' grains (Missotten et al., 2010; Sugiharto et al., 2015b). In most cases, the SmF technique is applied to produce fermented liquid feed (FLF) where the compound feed is fermented after mixing with water or liquid by-products from the food industry (Canibe and Jensen, 2003). The SmF approach is also a dominant technique in the propagation of probiotics in research and industry (Shim et al., 2010).

To date, studies on poultry fermented feeds have employed both SSF (Chiang et al., 2010; Cao et al., 2012; Tang et al., 2012; Sun et al., 2013b; Supriyati et al., 2015) and SmF methods (Mathivanan et al., 2006; Ao et al., 2011; Missotten et al., 2013) depending on the substrate types. However, SSF has received more interest as this method generates higher yields and better product characteristics than SmF (Couto and Sanroman, 2006; Shim et al., 2010). Indeed, the high water content in fermented feeds (porridge-like consistency) prepared by SmF, may be challenging for broiler production, in terms of practical feeding and litter quality (Engberg et al., 2009). Table 1 shows the advantages and disadvantages of SSF over SmF.

3. Characteristics of fermented feed

It is accepted that fermentation improved microbial and nutritional characteristics of particular feedstuffs (Canibe and Jensen, 2012; Khempaka et al., 2014; Sugiharto et al., 2015a). A study of fermented maize kernels showed that organic acids increased after

10 • 1 Advantages and disadvantages of solid state fermentation over submerged fermentation.¹

| Advantages | Disadvantages |
|---|--|
| - Higher productivity and low-cost media. | - Difficulties on scale-up. |
| - Better oxygen circulation. | - Low mix effectively. |
| - Less effort in downstream processing. | - Problems with heat build-up. |
| - Resembles the natural habitat for several microorganisms. | - Difficult control of process parameters (pH, heat, moisture, nutrient conditions, etc.). |
| - Simple technology and rare operational problems. | - Higher impurity product. |
| - Less energy and cost requirements. | - Increasing recovery product costs. |

¹ Adapted from Couto and Sanroman (2006).

fermentation, while the pH decreased from 5.5 to 4.2 coliform bacteria. Lactose negative enterobacteria, yeasts and moulds counts decreased significantly from approximately 6 to 3 log cfu/g after fermentation, but LAB counts increased to 8.2 from 7.5 log cfu/g (Ranjitkar et al., 2016). In general, fermented products tend have higher and lower numbers of LAB and *Enterobacteriaceae*, respectively, higher concentrations of organic acids (mainly lactic acid) lower pH values, when compared to raw material (Table 2). These properties make fermented feeds particularly beneficial for healthy gastrointestinal functions and well-being of chickens (Engberg et al., 2009; Sugiharto et al., 2016a,b). In addition to improved microbiological metrics, fermentation has been shown to decrease mycotoxins in feedstuffs. Okeke et al. (2015) showed that fermentation reduced mycotoxins in unsteeped maize grains, while Yang et al. (2018) reported reductions in zearalenone (mycotoxin produced mainly by *Fusarium*) under SSF conditions. It would appear that bacteria involved in fermentation play pivotal roles in the degradation and bio-transformation of mycotoxins to non-toxic compounds (Okeke et al., 2015).

Apart from these key antimicrobial properties, fermented feeds also contain acetic acids and biogenic amines (e.g. cadaverine, putrescine, and histamine) that potentially impair feed palatability (Canibe and Jensen, 2012). Indeed, fermentation has been shown to compromise some feed nutritional components, e.g. degradation of free lysine which may negatively affect host performance (Canibe and Jensen, 2003). Improving fermentation conditions/environments and/or adding acidifiers (e.g. organic acids) or concentrated starter LAB strains or enzymes, are often recommended to not only speed up fermentation processes, but also to improve nutritional

Table 2
The pH, microbial and organic acids composition of non-fermented feed, fermented dry feed and fermented liquid feed.¹

| Item | Non fermented feed | Fermented dry feed | Fermented liquid feed |
|-------------------------------|--------------------|--------------------|-----------------------|
| pH | – | 3.85 | 4.45 |
| LAB, log cfu/g | 4.3 | 9.4 | 9.6 |
| Yeasts, log cfu/g | <3.0 | 8.0 | 7.2 |
| Enterobacteriaceae, log cfu/g | 5.4 | <3.0 | <3.5 |
| Acetic acid, mmol/kg | 10 | 15 | 24 |
| Total SCFA, mmol/kg | 5.8 | NM | 38.4 |
| Lactic acid, mmol/kg | 8.0 | 50 | 160 |

NM = not measured; LAB = lactic acid bacteria; SCFA = short chain fatty acids.

¹ Modified from Canibe et al. (2007).

values and the palatability of the final fermentation product (Canibe and Jensen, 2012). To avoid losses of some essential nutrients in fermented feeds, Sugiharto et al. (2015b) suggested fermenting the grain fraction only (before incorporation into compound diets) instead of the complete diets.

Contamination of poultry feeds during storage, for example by bacteria, moulds and fungi, may cause spoilage which adversely affects feed quality as well as increasing poultry risks towards infections. High concentration of lactic acid and low pH in fermented feeds have been suggested to inhibit the growth of bacteria such as *Salmonella typhimurium* and *Escherichia coli* in the chicken diet (Murry et al., 2004; Niba et al., 2009). In accordance with this, Heres (2004) observed less *Campylobacter* and *Salmonella* contaminations in fermented feeds for chickens. Moreover, Londero et al. (2014) reported that the addition of fermented whey increased the resistance of poultry diets to fungal contamination. In conjunction with reduced bacterial/fungal contamination, the presence of metabolites, such as organic acids and bacteriocins (produced by LAB during fermentation) may have preservative effects and may increase the shelf-life of fermented feeds (Borresen et al., 2012; Londero et al., 2014).

4. Unconventional fermented feedstuffs

In most circumstances, high fibre and low protein contents limit the use of unconventional feed ingredients in broiler diets. One possible strategy to improve the quality of these feedstuffs for broiler diet eligibility is through the fermentation approach. As mentioned previously, fermentation may enhance the nutritional quality of feedstuffs by a number of processes: 1) lowering the fibre content (Skrede et al., 2003; Shahowna et al., 2013; Khiraka et al., 2014; Sugiharto et al., 2015a), 2) increasing crude protein and lipid content, 3) improving vitamin availability and 4) improving protein solubility and amino acid patterns (Agrahar-Murugkar and Subbulakshmi, 2006; Wang et al., 2010; Borresen et al., 2012). Fermentation has also been shown to increase the digestibility of various nutrients such as organic matter, nitrogen, amino acids, fibre and calcium (Canibe and Jensen, 2012), and to increase feedstuff palatability (Wang et al., 2010; Shahowna et al., 2013).

Another key aspect that must be considered when using novel feed ingredients for broiler diet is the presence of ANF that may impact digestibility and the nutritional value of feedstuffs. Fermentation has been reported to reduce ANF content in feed ingredients, e.g., lectins and trypsin inhibitors in soybean meal (Feng et al., 2007), glucosinolates in rapeseed meal (Chiang et al., 2010), tannins, haemagglutinins and prosopine in prosopis seed meal (Yusuf et al., 2008) and phytate in maize (Sokrab et al., 2014). These observations suggest that fermentation could increase the nutritive values of unconventional feed ingredients for broilers, by decreasing ANF content. Most fermentation mechanisms that reduce ANF contents are uncharacterized, but for phytates in maize, the work of Sokrab et al. (2014) has suggested that phytase enzymes produced by microorganisms during fermentation may degrade phytates leading to decreased phytate levels in the substrates. The presence of toxic compounds, such as cyanogenic glycoside compounds, phorbolsters, isothiocyanates, etc. pose other problems associated with the use of unconventional feedstuffs in the broiler diet. Fermentation could be a simple means to detoxify these toxins, as reported by Kobawila et al. (2005) and Suranindyah and Astuti (2012); these authors reported that the cyanide content of cassava roots and cassava peel, respectively, were diminished substantially after fermentation. Correspondingly, the phorbolster content of *Jatropha curcas* kernel cake (Belewu and Sam, 2010) and isothiocyanate content in rapeseed meal

(Chiang et al., 2010; Xu et al., 2012) decreased significantly after fermentation. With regards to cyanide reduction in cassava, Tefera et al. (2014) reported that linamarase, hydroxynitrile lyase and cyanide hydratase enzymes produced by microorganisms during fermentation, appeared to be responsible for hydrolysing linamarin, which is a cyanogenic glucoside.

5. The influence of fermented feed on growth performance

Feed ingredients such as wheat, soybean, barley and rapeseed contain considerable amounts of non-starch polysaccharides (NSP) that cannot be digested by poultry due to a lack of endogenous hydrolysing enzymes. One example is the rapeseed meal/cake, which is rich in protein and contains up to 400 g/kg crude protein (Bourdon and Aumaitre, 1990; Jakobsen et al., 2015). However, a major disadvantage of this feedstuff as a feed item for monogastric animals, e.g. chickens is the high NSP content, which is approximately 18–23% (235 g/kg) (Jakobsen et al., 2015). Indeed, soluble NSP increases digesta viscosity and reduces nutrient digestibility in the chicken small intestine (Choct, 1997). Feeding trials with rapeseed meal, fermented with *Lactobacillus fermentum* and *Bacillus subtilis*, showed improved weight gain and feed conversion ratio (FCR) in broiler chickens (Chiang et al., 2010). However, in another study, no health influences on broiler performance were observed by Xu et al. (2012) when soybean was replaced by up to 10% rapeseed meal fermented with *L. fermentum* and *B. subtilis*. Fermentation of other feedstuffs, e.g., *Aspergillus oryzae* fermented soybean meal (Feng et al., 2007; Mathivanan et al., 2006) and lactobacilli-fermented wheat and barley (Skrede et al., 2003) resulted in improved broiler performances when compared to broilers fed on non-fermented control diets. As demonstrated by Skrede et al. (2003), weight gain of broilers was higher by 182 to 232 g when fed fermented barley, and higher by 50 to 108 g when fed fermented wheat than that of broilers fed corresponding control diets. The authors also reported 29% lower soluble β -glucans after barley fermentation, but this was increased by 12% for wheat. In line with this observation, Skrede et al. (2003) suggested that the degradation of β -glucans in barley during fermentation was mainly associated with greater influences of fermented barley on body weight, when compared to fermented wheat. Moreover, increased weight gain and reduced FCR was observed in broilers when fed fermented barley and oats, believed to be due from a reduction of soluble NSP (β -glucan) of approximately 62% in barley and 40% in oats (Svihus et al., 1995, 1997). This suggests that improvement in nutritive values and the digestibility of feed ingredients through soluble NSP degradation during fermentation is a major factor underpinning improved broiler performances.

However, Svihus et al. (1997) further reported that similar results were not observed when broilers were fed fermented wheat, most probably due to the presence of low soluble NSPs in wheat (5.1 to 6.6 g/kg) as compared to barley (53 to 61 g/kg) and oats (31 to 46 g/kg). Furthermore, results from Jakobsen et al. (2015) showed that the addition of enzyme mixtures (glucanase, xylanase and pectinase) during fermentation was highly effective in reducing total NSP by 31% to 42% in rapeseed cakes, as no reduction of NSP was observed after fermentation process in lack of enzymes. In a composite flour (soybean, maize and germinated sorghum) fermented with yeast (*Saccharomyces cerevisiae*) for 24 h, it was observed that crude protein content increased slightly from 252 to 275 g/kg dry matter (Murekatete et al., 2012). The authors further reported increase in *in vitro* protein digestibility (12%) of the fermented composite flour. Likewise, crude protein content in rapeseed meal increased marginally from 387 to 423 g/kg when fermented with *S. cerevisiae* for 24 h (Jaiapetch and Yakupitiyage, 2012). Owing to the fact that fermentation may improve

nutritional quality, digestibility and palatability of unconventional feed ingredients, the inclusion of these feedstuffs after fermentation in poultry ration can reasonably be expected to reduce feed costs without impairing the bird performance. Table 3 shows examples of conventional and unconventional fermented feedstuffs and their positive influences on the growth performance of broiler chickens. However, the results are not consistent.

Recent studies on crimped kernel maize silage (CKMS) demonstrated no significant differences in body weight gain of broilers, between the control maize group and the 15% CKMS group (Ranjitkar and Engberg, 2016; Ranjitkar et al., 2016a), although counts of LAB were higher (8.2 versus 7.5 log cfu/g) and pH was lower (4.2 versus 5.5) in the experimental diet when compared to the control diet. Furthermore, the authors reported decreased body weight in broilers when CKMS was increased to 30% (Ranjitkar

et al., 2016a). Likewise, feeding unconventional feedstuffs such as palm kernel cake (Alshelmani et al., 2016), or cassava pulp (Khempaka et al., 2009) to broilers has typically been associated with impaired growth performances, when compared with feeding conventional soybean meal.

Apart from improved nutritional metrics and increased digestibility from fermentation processes, the underlying mechanisms of why fermented feeds promote growth performances of broilers is largely unknown. Some studies have suggested that fermented feeds increase intestinal length indices to maintain normal gut microbial ecosystems and improve intestinal morphology (e.g., villus height and villus height to crypt depth [VH:CD] ratio in the duodenum and jejunum). Fermentation feeds may be responsible for improvements in digestion and absorption which in turn improve production performance of birds (Feng et al.,

Table 3
Examples of fermented conventional and unconventional feedstuffs and their effects on the performance of broiler chicken.

| Fermented feed | Results | References |
|--|---|--------------------------|
| Fermented compound feed (Bactocell as starter inoculum) | Improved feed intake and weight gain of broiler chickens. | Uche wa Onu (2012) |
| Wet fermented feed (feed water ratio, 1:1.2 to 1:1.4) | Increased weight gain and improved feed conversion of hens compared to the hens fed dry mash. | Engberg et al. (2009) |
| <i>Aspergillus oryzae</i> 3.042-fermented soybean meal | Increased average daily gain and feed intake of the chicks as compared with those fed non-fermented soybean meal. | Feng et al. (2007) |
| <i>A. niger</i> -fermented soybean meal | Increased body weight of broiler at 5th and 6th weeks of age compared to control when administrated at 0.5% from diet, but the effect did not last when the fermented soybean meal was included at 1% and 1.5%. | Mathivanan et al. (2006) |
| Fermented wheat and barley (no microbial inoculants) | Increased body weight of broilers at 42 and percentage of carcass yield. | Yaşar et al. (2016) |
| Fermented barley or wheat (<i>Lactobacillus</i> or <i>Bacillus</i> as starter inoculums) | Improve growth performance of broiler chickens. | Kim and Kang (2016) |
| <i>A. niger</i> and <i>Candida utilis</i> -fermented olive leaf residue | Supported the chicken growth and development when administrated at 10% from diet. | Xie et al. (2016) |
| Fermented dried cassava (spontaneous fermentation) | Had no negative impact on the growth performance of broilers when provided at up to 10% from diets. | Sugiharto et al. (2016b) |
| Crimped kernel maize silage | Had no adverse effect on growth performance of 37-day-old broilers when administrated at 15%, but inclusion at 30% decreased broiler growth. | Ranjitkar et al. (2016a) |
| <i>A. oryzae</i> -fermented cassava pulp | Had no detrimental effect on nutrient digestibility and growth performance of broilers when included at up to 16%, but decreased nutrient digestibility and growth when used at 20% and 24% from total diets. | Khempaka et al. (2014) |
| <i>Bacillus subtilis</i> and <i>A. oryzae</i> -fermented seaweed | Improved the weight gain and feed:gain ratio of the birds compared to control. | Choi et al. (2014) |
| <i>A. niger</i> -fermented <i>Ginkgo biloba</i> leaves | Attenuated the decrease of daily gain and feed intake due to lipopolysaccharides (LPS) challenge. | Zhang et al. (2013) |
| <i>B. subtilis</i> BJ-1-fermented cottonseed meal | Increased the body weight gain of broilers in the starter and in the overall feeding periods when included at 40 and 80 g/kg in diet. | Sun et al. (2013a) |
| <i>B. subtilis</i> BJ-1-fermented cottonseed meal | Improved the weight gain and feed intake of broiler when administrated at 8% from total diet. | Tang et al. (2012) |
| <i>Lactobacillus fermentum</i> and <i>B. subtilis</i> -fermented rapeseed meal | Had no adverse effect on the performance of broiler when included in the diet up to 10% (to replace soybean meal), but inclusion at 15% decreased body weight gain of broilers. | Xu et al. (2012) |
| <i>A. niger</i> and <i>Penicillium chrysogenum</i> -fermented mango kernel cake | Had no detrimental effect on the performance of broiler when it was included in the diet up to 27% (to replace the maize). | Kayode et al. (2012) |
| <i>A. niger</i> -fermented <i>Terminalia catappa</i> fruit meal | Had no adverse effect on the feed intake, weight gain, and feed:gain ratio compared to control when included up to 18% (to replace the maize) in the diet of broiler. | Apata (2011) |
| <i>Bifidobacterium</i> H-1-fermented ginseng extract | Had no impact on the performance of broiler chicken and laying hen. | Ao et al. (2011) |
| Fermented rapeseed meal (<i>L. fermentum</i> , <i>Enterococcus faecium</i> , <i>Saccharomyces cerevisiae</i> and <i>B. subtilis</i> prepared in a 1:1:1:1 ratio were the starter) | Improved weight gain and feed conversion compared to the broiler fed non-fermented rapeseed meal, and did not differ from the soybean control. | Chiang et al. (2010) |
| <i>A. niger</i> -fermented cassava leaf meal product | Had no harmful effect on the performance of broiler when included in the rations up to 25%. | Widjastuti et al. (2010) |
| Fermented potato pulp (LAB and yeast isolated from potato pulp was the starter) | Had no detrimental effect on the daily gain and feed:gain in quails as compared to control. | Wang et al. (2010) |
| <i>S. cerevisiae</i> fermentation product | Improved the daily gain of the chicken after coccidian infection. | Gao et al. (2009) |
| <i>Lactobacillus plantarum</i> -fermented rice bran | Increased the weight of eggs. | Loh et al. (2007) |
| <i>Lactobacillus</i> strain AD2-fermented barley and wheat | Improved growth and early feed:gain ratio of broiler chickens compared to those fed non-fermented barley and wheat. | Skrede et al. (2003) |
| Fermented cassava peel meal | Improved growth rate and feed efficiency of the birds as compared to those fed control diet. | Osei and Duodub (1988) |

LAB = lactic acid bacteria.

2007; Chiang et al., 2010; Missotten et al., 2013; Sun et al., 2013b; Zhang et al., 2016). Another study suggested that positive influences of fermented feeds seen in gastrointestinal environment/condition (e.g., the lowering of gastric pH and pathogenic microbial activity as well as increasing the production of short chain fatty acids [SCFA]), may be attributable to the increased digestibility of feeds, which in turn improves the growth performance of chickens (Mathivanan et al., 2006). Moreover, Feng et al. (2007) and Sun et al. (2013a) revealed that increased activities of digestive enzymes such as amylases, trypsin, lipases and proteases in broilers on fermented feeds, are responsible for growth improvements in birds.

Although fermentation is associated with improved palatability (Supriani et al., 2015) and nutrient digestibility (Alshelmani et al., 2016), Missotten et al. (2013) reported that fermentation decreased the feed intake of broilers during starter and grower phases, when compared with controls. This resulted in retarded growth rates of chickens during these phases, but interestingly not in the finisher phase. Several rationales may explain these observations: 1) the moist diet may have been too bulky for small birds, 2) the diet may have lost its attractiveness after fermentation (24 h soaking) and 3) fermentation may cause a loss of essential nutrients (e.g. lysine) (Missotten et al., 2013). Concomitant with the previous study, Engberg et al. (2009) observed a lower feed intake in laying hens fed fermented feeds when compared to those fed dry mash. Nonetheless, hens receiving fermented feed had a greater body weight and no differences were observed with respect to total egg mass production between hens fed fermented feed and those fed dry mash. Altogether, fermented feed appears to be more beneficial for chicken performance when specifically fed during the late broiler phase, or during the laying period. In addition, fermentation could be a promising tool maximizing the use of unconventional feed ingredients for the poultry diet.

Other than growth performances, breast meat yields and abdominal fat deposition are other concerns when feeding unconventional diets to broiler chickens, as these parameters are often used as key measures of meat production (Widjastuti et al., 2010). In the study by Kayode et al. (2012), the inclusion of *Aspergillus niger* and *Penicillium chrysogenum*-fermented mango kernel cake, in up to 60% of the diet, had no impact on breast meat yields and abdominal fat deposition, when compared to controls. Similarly, the authors also showed that inclusion of fermented mango kernel cake, in up to 20% of the diet, did not impact carcass weight. A similar result was observed by Santoso et al. (2004), where the inclusion of *Lactobacillus* spp.-fermented (layer) faeces, in up to 15% of the diet, did not influence carcass weights and abdominal fat deposition in broiler chickens. Moreover, Widjastuti et al. (2010) reported that inclusion of *A. niger*-fermented waste-cassava leaf meal, of up to 25% of the diet, had no influence on carcass weights and carcass percentages. Mathivanan et al. (2006) also reported that inclusion of *A. niger*-fermented soybean meal, of up to 1.5% of the diet, had no impact on the ready-to-cook weight or the live weight of broilers.

Unlike these studies, lower breast meat yields (percentage of live body weight) were recorded in birds fed fermented moist feeds in the study by Missotten et al. (2013). In their study, although treatments did not affect the dressing percentage of broilers, Skrede et al. (2003) found that abdominal fat increased with increasing levels of *Lactobacillus* strain AD2-fermented barley in administered feeds. Likewise, Zhang et al. (2016) reported that the inclusion of 6% fermented feed into the diet resulted in increased abdominal fat percentages of 56-day-old broiler chickens. In contrast to these last two studies, Nie et al. (2015) reported that feeding cottonseed meal fermented by *Candida tropicalis* and *S. cerevisiae*, decreased abdominal fat relative weight (due to increased fatty acid β -oxidation and triglyceride hydrolysis) in

broilers. Overall, these differences in characteristics and proportions of fermented feed ingredients, may be responsible for discrepancies regarding the abdominal fat content of broilers fed fermented diets.

A previous study showed that feeding fermented feeds appeared to improve the fatty acid profiles of chicken meat. Marcincák et al. (2018) reported that feeding 10% cornmeal, fermented with *Umbelopsis isabellina* CCF2412, resulted in increased proportions of gamma-linolenic, alpha-linolenic and oleic acids in breast meat fat, and improved ratios of n-6 to n-3 polyunsaturated fatty acids in the raw meat. These authors also documented that fermented feed improved the quality, oxidative stability and sensory properties of broiler meat. Unlike this study, Chung and Choi (2016) reported that feeding 1% fermented red ginseng marc with red koji, did not affect fatty acid profiles in breast and thigh muscles of broiler chicks. It appears that the different nature and levels of fermented feeds, and the performance of *in vivo* trials may explain these divergent results.

6. Influence of fermented feed on gastrointestinal tract microecology

The intestinal microbiota is an integral part of the gastrointestinal tract and plays an important role in nutrition, physiology and gut morphology. In addition, the microbiota is involved in host immune defence mechanisms against pathogens. In pig nutrition, FLF is a tool that improves gut microbial ecosystems by reducing the colonization of enterobacteria such as coliforms and salmonella in the gut (Canibe and Jensen, 2003, 2012; Højberg et al., 2003). Similarly, fermented feeds could also be beneficial in maintaining healthy gastrointestinal ecosystems in poultry, owing to key characteristics such as low pH, high numbers of lactobacilli, high lactic acid and acetic acid concentrations and low enterobacteria numbers (Canibe and Jensen, 2003, 2012; Engberg et al., 2009). The advantages of a fermented feed diet on broiler gastrointestinal tract microecology is summarized in Table 4.

The beneficial influences of fermented feeds on broiler gastrointestinal tract microecology are due to individual feed characteristics (Heres et al., 2003a, b; Engberg et al., 2009; Sun et al., 2013a). The LAB present in fermented feeds not only reduces gut pH through the production of organic acids, but they also prevent the colonization of enteropathogens through competitive exclusion, antagonistic activities and bacteriocin production (Kierończyk et al., 2016). As previously discussed, administering fermented feed increases LAB populations in the chicken gut (Savvidou et al., 2009; Sun et al., 2013a). This is probably due to the acidifying effects of fermented feed on the gut, thereby providing a favourable environment for LAB colonization (Heres, 2004; Loh et al., 2007).

The low pH of fermented feeds acidifies the upper digestive tract and thereby improves the barrier function of the gizzard against pathogens. Fermented feeds also create unfavourable environments for the proliferation of certain enteropathogens like *E. coli* (Liang et al., 2012), *Salmonella* (Heres et al., 2003a; Heres, 2004) and *Campylobacter*, in broilers (Heres et al., 2003b). Fermented feeds using *Lactobacillus plantarum* as starter cultures were shown to reduce faecal counts of *Enterobacteriaceae* in laying hens (Loh et al., 2007), while fermented moist feeds decreased coliform counts and *Streptococci* in the small intestine of broiler chicks (Missotten et al., 2013). As stated, decreased bacterial number attributing of feeding fermented feed subsequently reduces the competition for easily available nutrients resulting in improved growth and broiler performance (Engberg et al., 2009). In the study by Ranjitkar and Engberg (2016) and Sharma et al. (2017), no differences in *Campylobacter jejuni* and *Clostridium perfringens* counts were reported between broilers who had received 15% to 30% CKMS, and

Table 4
Examples of fermented conventional and unconventional feedstuffs and their effect on the gastrointestinal tract microecology of broiler chicken.

| Fermented feed ¹ | Gastrointestinal tract microecology | References |
|--|---|--------------------------|
| Fermented feed | Increased acetic and propionate concentration in the ceca of broilers when administrated at the level of 4%. | Zhang et al. (2016) |
| <i>Lactobacillus plantarum</i> NCIMB 40087-fermented moist feed | Increased the number of lactobacilli in the foregut, and decreased the number of coliform in the foregut and streptococci in ileum and caeca. | Missotten et al. (2013) |
| Fermented complete formula feed | Reduced the counts of <i>E. coli</i> in chicken jejunum, and increased the number of LAB. | Liang et al. (2012) |
| <i>Lactobacillus salivarius</i> -fermented liquid feed | Increased the counts of lactobacilli and production of lactic acid, and reduced the pH in the gut of chicken. | Savidou et al. (2009) |
| Wet fermented feed | Reduced proportion of <i>Salmonella typhimurium</i> in the gut of chicken. Lowered the counts of coliform bacteria in gut digesta of hens as compared to those of fed dry mash. | Engberg et al. (2009) |
| Fermented feed | Increased concentrations of SCFA and lactic acid in the contents of crop and gizzard than hens fed with dry feed. | Heres (2004) |
| Fermented liquid feed (<i>Lactobacillus</i> spp. was the starter) | Stabilized the gastrointestinal microbiota of the chicken. Modulated the composition of microbiota in the crop, jejunum and caecum by increasing the number of lactobacilli. | Koenen et al. (2004) |
| Fermented liquid feed (<i>L. plantarum</i> was the starter inoculum) | Decreased <i>Salmonella enteritidis</i> colonization in broiler chickens. Inhibited the introduction of <i>Salmonella</i> in broiler flocks. | Heres et al. (2003b) |
| Palm kernel cake fermented by <i>Paenibacillus polymyxa</i> ATCC 842 | At 15% from total diet, increased and decreased LAB and Enterobacteriaceae counts, respectively, in the intestine of broilers. | Alshelmani et al. (2016) |
| Fermented barley or wheat (<i>Lactobacillus</i> or <i>Bacillus</i> were used as fermenters) | Increased <i>Lactobacillus</i> spp. population in the intestine of broilers, but no effect on the number of <i>E. coli</i> . | Kim and Kang (2016) |
| Fermented cottonseed meal | Increased the number of lactobacilli in the caecal digesta and decreased coliform bacteria. | Sun et al. (2013a) |
| Fermented rapeseed meal | Increased the number of lactobacilli in the colon and caecal digesta as compared to broilers fed the control and non-fermented rapeseed meal diets. | Chiang et al. (2010) |
| Silage (maize or barley-pea silage) as supplement for hens | Reduced the pH in gizzard-content of hens. Increased the concentrations of lactic acid and acetic acid in the gizzard contents of hens. | Steenfeldt et al. (2007) |
| Fermented product based on rice bran, fish, lime, molasses and vinegar | Reduced the faecal numbers of Enterobacteriaceae and pH value, and increased the number of LAB in the gut of laying hens. | Loh et al. (2007) |

NCIMB = National Collections of Industrial, Marine and Food Bacteria; LAB = lactic acid bacteria; SCFA = short chain fatty acids; ATCC = American Type Culture Collection.

¹ The starter inoculums that have been mentioned in Table 3 (with the same references) were not stated in Table 4.

the control groups. Likewise, gut bacterial diversity did not significantly vary between broilers fed maize-based diets and diets supplemented with 15% to 30% CKMS (Ranjitkar et al., 2016b). However, the inclusion of 30% CKMS lowered the proliferation of potentially pathogenic bacteria such as coliforms (Sharma et al., 2017). It is accepted that normal intestinal microbiota are essential for controlling intestinal colonization by pathogens, and that this normal microbiota minimizes the risks of enteric disease outbreaks in broilers. In this regard, reducing infection vulnerabilities in flocks may require the strategic feeding of fermented feed to broilers as an effective strategy in reducing the transmission of chicken enteric diseases (Heres et al., 2003a,b).

7. Influence of fermented feed on gut morphology

Healthy functioning of the intestine is essential for the growth performance and well-being of broilers. The intestinal mucosa plays key important roles in the digestion and absorption of dietary nutrients, it protects the sterile internal milieu from hostile luminal contents and it defends against harmful dietary substances and pathogens (Sugiharto et al., 2015c). It has been reported that VH:CD ratio is an important parameter for the estimation of absorptive capacity of the small intestine in chickens, and the absorption of nutrients increases with increasing VH:CD ratio (Mathivanan et al., 2006; Chiang et al., 2010). Importantly, study has connected the diet and the maintenance of intestinal mucosal integrity (Jankowski et al., 1994). Of the reviewed diets, the fermented feed diet has been reported to improve villus height and VH:CD ratio, in the jejunum and ileum of chickens (Table 5). Different from those of reported in Table 5, Alshelmani et al. (2016) reported that feeding *Paenibacillus polymyxa* ATCC 842-fermented palm kernel cake, did

not affect the intestinal morphology of broiler chickens, although such treatments increased intestinal LAB population in chickens.

The improvement of mucosal morphology of chickens fed fermented feed has typically been associated with an increase in the numbers of lactobacilli in the intestine (Chiang et al., 2010). Similarly, Missotten et al. (2013) suggested that feeding fermented feed may positively modulate the composition of intestinal bacteria, which in turn prevents excessive inflammatory responses against pathogens in the intestine, resulting in improved histomorphology indices. The lower levels of toxins and antigenic materials found in fermented feed has also been suggested to benefit the intestinal morphology of broiler chickens (Chiang et al., 2010; Xu et al., 2012). A study in pigs, by Wang et al. (2003), reported that adding small peptides to basal diets improved intestinal morphology as indicated by greater villus heights in the duodenum, jejunum and ileum and lower crypt depths in these tissues. In this respect, increased levels of small peptides in the fermented feed (Tang et al., 2012; Xu et al., 2012) appeared to favourably influence broiler intestinal morphology. Moreover, reduced ANF, such as trypsin inhibitors, in fermented feed may positively affect chicken intestinal morphology, as reported by Feng et al. (2007).

8. Influence of fermented feed on the immune system

The restricted use of antibiotic growth promoters in poultry feeds has encouraged more nutrition-based research to determine alternative feed sources to enhance the immune competence of chickens. Many feed ingredients have the potential to improve the immune responses of chickens (Sugiharto, 2016). Although only limited data are available, feeding fermented feed has been shown to decrease broiler mortality rates (Ranjitkar et al., 2016a) and

Table 5
Examples of fermented conventional and unconventional feedstuffs and their effect on the gut morphology of broiler chicken.

| Fermented feed ¹ | Gut morphology | References |
|---|---|--------------------------|
| Fermented feed | Increased intestinal length index, villus height as well as crypt depth of ileum of broilers at the level of 4% and 6%. | Zhang et al. (2016) |
| <i>Lactobacillus plantarum</i> NCIMB 40087-fermented moist feed | Improved the villus height in the mid-jejunum and mid-ileum as compared to control birds. | Missotten et al. (2013) |
| Fermented cottonseed meal | Increased villus height in the duodenum and elevated villus height and VH:CD ratio in the jejunum of broiler. | Sun et al. (2013a) |
| Fermented- <i>Ginkgo biloba</i> leaves | Attenuated the decrease in duodenal and jejunal relative weights, villus height, crypt depth after challenge with LPS. | Zhang et al. (2013) |
| Fermented rapeseed meal | Up to 10% to replace soybean meal, increased villus height: crypt depth ratio in the jejunum of broiler. | Xu et al. (2012) |
| Fermented rapeseed meal | Improved villus height and VH:CD ratio in the ileum and jejunum of broiler. | Chiang et al. (2010) |
| Fermented soybean meal | Increased villus height of duodenum and jejunum and decreased crypt depth of the jejunum mucosa of broiler. | Feng et al. (2007) |
| Fermented soybean meal | Lowered the pH of intestine and improved the ileal villi length and width (at 0.5% and 1% from diet) compared to control. | Mathivanan et al. (2006) |

VH:CD ratio = villus height to crypt depth ratio; LPS = lipopolysaccharides.

¹ The starter inoculums that have been mentioned in Tables 3 and 4 (with the same references) were not stated in Table 5.

positively affect broiler immune responses (Table 6). This feeding strategy not only induces circulating antibodies, but also increases the mucosal immunity of broiler chickens (Gao et al., 2009). Moreover, fermented feeds have been associated with decreased heterophil to lymphocytes ratios in broilers (Kim and Kang, 2016). This observation suggested that fermented feeds alleviated oxidative stress in chickens, which could lead to immunosuppression (Sugiharto et al., 2016c). Several studies observed the formation of small sized peptides during fermentation which were believed to be associated with increased immunoglobulin (Ig) levels in birds (Tang et al., 2012; Xu et al., 2012). This inference was reasonable based on a pig study by Wang et al. (2003). These authors supplemented 3 g of small peptides/kg of the pig basal diet and recorded increased Ig concentrations in piglet serum.

It has been observed that changes in the composition of bacteria along the gut may affect the immune responses of chickens (Missotten et al., 2013). The feeding of fermented diets, which is typically associated with increased LAB populations in broiler guts (Table 4), appears to favourably affect the immune functions of these chickens. The exact LAB mediated immunomodulatory

activities in broilers are as yet unclear, however they may stimulate different subsets of immune cells to produce cytokines, which play key roles in the induction and regulation of immune responses. In his review, Kabir (2009) suggested that LAB, especially lactobacilli, induces Th2 cytokine production, such as the interleukins IL-4 and IL-10, which promote B cell development and Ig isotype switching required for antibody production.

In addition to antibody-mediated immune responses, feeding fermented diets has also been demonstrated to increase cell-mediated immune responses in chickens (Xi-jie et al., 2007; Gao et al., 2009). The high LAB content in fermented feeds appears to be responsible for these physiological observations. In a mice study by Parvinder and Aruna (2012), it was shown that *Lactobacillus acidophilus* exerted direct influence on Th1-cell immunity by modulating the levels and activities of antigen-specific T lymphocytes and/or could exert an indirect influence on T-lymphocyte activity through stimulation of other cell types, such as phagocytes. A study in pigs showed that feeding fermented diets was associated with increased intestinal SCFA concentrations (Canibe and Jensen, 2003). These fatty acids have been recognized as potential

Table 6
Examples of fermented conventional and unconventional feedstuffs and their effect on the immune responses of broiler chicken.

| Fermented feed ¹ | Immune function | References |
|---|---|----------------------|
| Fermented feed | Increased plasma IgG and S-IgA (Secretory IgA) in duodenum of broilers when administrated at the level of 4% and 6% from diet. | Zhang et al. (2016) |
| <i>Saccharomyces cerevisiae</i> fermentation product | Increased CD3 ⁺ , CD4 ⁺ and CD8 ⁺ lymphocyte counts and CD4 ⁺ :CD8 ⁺ ratio in the blood and spleen as well as ileum intraepithelial lymphocyte count, caecal tonsil secretory IgA counts, serum lysozyme content and albumin:globulin ratio of broilers. | Gao et al. (2009) |
| Fermented feed (EM/commercial microbial additive was the starter) | Increased the antibody concentration and T cells of birds. | Xi-jie et al. (2007) |
| Fermented liquid feed | Enhanced IgM and IgG responses to TNP. | Koenen et al. (2004) |
| Fermented soybean meal | Increased the serum concentration of IgM in the whole period and IgA in the growing period of broiler compared to broilers fed control diet (non-fermented soybean meal). | Feng et al. (2007) |
| Fermented seaweed | Increased the concentrations of IgM and IgA in the serum, but decreased IgG when compared to that in control. | Choi et al. (2014) |
| Fermented- <i>Ginkgo biloba</i> leaves | Decreased the duodenal and jejunal interferon (IFN)- γ , interleukin (IL)-4, IL-13, IL-18, inducible nitric oxide synthase and duodenal sodium glucose cotransporter 1 mRNA expression levels in LPS-challenged birds. | Zhang et al. (2013) |
| Fermented cottonseed meal | Increased serum IgM, IgG and complement C4 levels compared with bird fed control diet. | Tang et al. (2012) |
| Fermented rapeseed meal | Increased the serum concentration of IgG and IgM in broiler chicken. | Xu et al. (2012) |
| Fermented red ginseng extract | Improved the lymphocyte level compared to control. Increased the weight of bursa of Fabricius and spleen of the chicken. | Ao et al. (2011) |

EM = effective microorganism; TNP = trinitrophenyl; LPS = lipopolysaccharides.

¹ The starter inoculums that have been mentioned in Tables 3–5 (with the same references) were not stated in Table 6.

mediators involved in intestinal immune functions, e.g., they regulate several leukocytic functions including the production of cytokines (tumour necrosis factor [TNF]- α , IL-2, IL-6, and IL-10), eicosanoids and chemokines (Vinolo et al., 2011). From these observations it is reasonable to assume that animals fed fermented diets experienced improved immune functions.

Apart from improved circulatory and intestinal immunity as previously discussed, a feeding diet containing fermented ingredients for chickens, was found to increase the weight of immune organs, which may reflect the immune status of the birds (Ao et al., 2011; Liang et al., 2012). However, Tang et al. (2012) and Choi et al. (2014) showed no significant changes in the relative weights of the spleen, thymus, and bursa of Fabricius in broilers after feeding with fermented cottonseed meal and fermented seaweed, however, interestingly, total serum Ig increased. Similar to the previous study, Kayode et al. (2012) reported that feeding fermented mango kernel cake had no impact on spleen weight from broiler chickens. Different study characteristics, experimental approaches and levels of fermented feeds may explain these discrepancies in immune organ weights.

9. Influence of fermented feed on welfare

An important reason for the limited use of fermented feeds in broiler diets is because of the assumption that such diets may cause wet litter, which in turn affects the behaviour and well-being of chickens (Missotten et al., 2013). Skrede et al. (2003) reported that litter quality was significantly reduced as the levels of fermented wheat or barley in chicken diets increased. However, Missotten et al. (2013) observed no visual differences in litter conditions between birds fed fermented and non-fermented feeds. Indeed, Ranjitkar et al. (2016) reported that feeding crimped kernel maize silage, improved the litter quality in terms of dry matter content, and a lower frequency of broiler foot pad lesions.

In terms of the influence of fermented feed on broiler welfare, there is a paucity of data. In the study by Steinfeldt et al. (2007), feeding silage (maize or barley-pea silage) as a supplement for hens, reduced feather pecking behaviour and improved plumage quality. In contrast, Engberg et al. (2009) reported that feeding wet fermented feed (feed to water ratio, 1:1.2 to 1:1.4) resulted in more aggressive behaviours and poorer plumage conditions, when compared to birds given dry feed. The characteristic of some fermented feeds given to birds, in terms of water content, and other parameters such as environmental conditions during rearing periods may explain these contrasting results. More studies must be conducted to elucidate the influences of fermented feed on broiler behaviour and welfare.

10. Conclusions

Fermentation is an inexpensive means to improve the nutritional value of novel unconventional feed ingredients for broiler chickens. It is apparent that fermented feed exhibits beneficial influences on gut ecosystems and morphology, immune functions as well as growth performance of birds. Fermented feeds of low pH and high LAB and lactic acid contents play major roles in determining positive influences on gut health and performance. Thus, fermented feeds could be applied as strategic tools to minimize the colonization of gastrointestinal pathogens in broilers. However, diets are diverse from one study to the other, in term of substrates, microbiota, and inclusion levels of fermented products, nutritional composition and water content. Furthermore, studies have been performed in different geographical areas of the world with varying environmental conditions, during key rearing period. In accepting this variability, it is therefore difficult to compare these inconsistent

and contrasting findings. However, on balance, fermentation appears to be an effective technique in upgrading the nutritional values of unconventional feed ingredients. Therefore, the use of locally available fermented feed ingredients, especially in developing countries could decrease feed costs, ensuring profitable broiler production. While data on the application of fermented feed for broiler production are limited, future studies must be consistent in their study approaches and they must be mindful how fermented feed components affect the microbiota of the broiler populations they are investigating.

Conflict of interest

The authors declare that they have no competing interests.

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