ORIGINAL ARTICLE

EFFECT OF BOVINE LACTOFERRIN ON GROWTH PERFORMANCE AND INTESTINAL HISTOLOGIC FEATURES OF BROILERS

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ABSTRACT

A 42-day feeding trial was conducted to determine the effect of bovine lactoferrin (bLf) on the performance and intestinal histological features of broilers. Three hundred straight-run day-old Cobb broilers were distributed to five treatments following a completely randomized design: basal diet without bLf or chlortetracycline (CTC), diet with 1 g CTC 15%/kg feeds and diets with 130, 260 and 520 mg bLf/kg feed offered during the starter stage. Metabolizability of nutrients, performance parameters and intestinal villi height and crypt depth were determined. Nutrient digestibility and apparent metabolizable energy of feeds with 130 and 260 mg bLf/kg feed were the same (P>0.05) with the CTC-supplemented feeds. Bovine Lf did not affect (P>0.05) the feed intake and livability of birds. Body weight gain and feed conversion ratio were improved (P<0.05) when bLf was added in the diet and were comparable (P>0.05) to broilers fed diets with CTC. Villi height increased (P<0.05) as bLf was increased in the diet up to 260 mg/kg feed, but decreased at 520 mg/kg feed which was comparable to CTC-fed birds. Crypt depth improved (P<0.05) when broilers were fed diets with either CTC or bLf. Results suggested that bLf can be used as replacement for CTC.

Keywords: broiler, lactoferrin, metabolizability, performance, villi height

INTRODUCTION

The use of in-feed antibiotics is effective in improving the general performance of the animal by preventing the proliferation of harmful microorganisms in the gut, thus reducing microbial metabolites and decreasing competition for nutrients. Extensive use of these antibiotic growth promoters (AGP), such as chlortetracycline, had attracted public concern regarding safe consumption of animal products. Some of these issues include antibiotic resistance, residue in animal products and increased production cost. This global challenge had pushed banning of AGPs in some countries and has driven research institutions, feed manufacturers and poultry farms to search for alternatives. Alternatives to AGP include antimicrobial proteins, organic acids, mineral clays, probiotics, prebiotics, essential oils, exogenous enzymes and immune-stimulants. The most abundant antimicrobial proteins are the lysozyme, collectin and lactoferrin. They are produced by a wide variety of organisms as their first line of defense and are found in large quantities in all secretory fluids.

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Lactoferrin (Lf) is 80 kDa glycosylated protein with 700 amino acids with high homology among species. It is a multifunctional protein known to work as an opsonin to promote bacterial clearance (Jensen and Hancock, 2008). Broilers fed diet with rice expressing lysozyme and Lf had lower feed intake and improved feed efficiency (Humphrey *et al.,* 2002). This improvement was attributed to the increased villi length, and consequently the surface area that would facilitate better nutrient digestion and absorption. Commercially available bovine lactoferrin (bLf) is commonly used in human food, skin and oral care (Wakabayashi *et al*., 2004). Moreover, when tried to weaned pigs, Wang *et al*. (2006) reported that supplementation with bLf improved final body weight by 13.5 % (P<0.05), increased average daily gain by 41.80% (P<0.01) and efficiency of gain by 17.20% (P<0.05). Intestinal villus height was increased by 15.30% (P<0.05), and crypt depth was decreased by 9.60% (P<0.05). These results were related to the functions of bLf which is an important component of the non-specific immune system and has antimicrobial, antifungal and antiviral properties. Since bLf could improve the performance of the animals, similar to what in-feed antibiotics contribute, bLf could have potential to replace AGP which causes antibiotic resistance to the animals. Therefore, this study was conducted to determine the effect of bovine lactoferrin (bLf) on the performance of broilers, energy and protein digestibility of broiler feeds and evaluate its effects on the intestinal histologic features of broilers.

MATERIALS AND METHODS

Materials

Commercial pharmaceutical grade bLf was used in the feeding trial which was 89.03% pure based on bLf enzyme-linked immuno-sorbent assay (ELISA-antigen sandwich) kit. Chlortetracycline (CTC) 15.00% was used to compare the performance of the birds receiving bLf with the performance of AGP.

A total of 300 day-old Cobb straight-run broilers were allocated to five dietary treatments in a completely randomized design for 42 days. Each treatment was replicated six times with ten chicks per replicate. The ingredients and calculated nutrient content of the diets are presented in Table 1. Booster feed was given from day 1 to 7, starter diet from day 8 to 28 and finisher diet from day 29 to 42. Feeds and water were available to the birds at all times. Artificial light was provided to allow the birds to eat and drink at night. The birds were vaccinated against NCD at days 7 and 21. Initial and body weights at days 28 and 42 were recorded to determine the average body weight gain. At 42 days of age, representative birds of each treatment were slaughtered. One bird from each replicate was randomly selected and dressed to determine the dressing percentage. The intestines from each slaughtered bird were collected and fixed in 10% formalin solution for histological examination.

Diets were formulated to meet the PHILSAN (2010) nutritional requirements of broilers and contained 22.30%, 20.00% and18.70% crude protein for booster, starter and finisher diets, respectively and metabolizable energy of 2,900 kcal/kg for booster and 2,800 kcal/kg both for starter and finisher diets. Bovine Lf was added to the starter ration of the birds. The dietary treatments were as follows:

Treatment	Description
1	Starter diet without bLf and CTC 15% (negative control)
2	Starter diet with 1g CTC 15%/kg feeds (positive control)
3	Starter diet with 130 mg bLf/kg feeds
4	Starter diet with 260 mg bLf/kg feeds
5	Starter diet with 520 mg bLf/kg feeds

Table 1. Ingredients and nutrient contents of the diets of birds fed bovine lactoferrin or chlortetracycline.

Ingredients	Booster	Starter	Finisher
Yellow corn	54.69	56.3	55.45
Soybean oil meal-Hi pro	37.03	30.87	27.02
Rice bran D1	3.00	9.00	14.00
Crude coconut oil	1.90	0.50	0.50
Limestone	0.90	1.00	0.80
Monodicalcium phosphate	1.60	1.50	1.40
Iodized salt	0.40	0.40	0.40
DL-methionine	0.15	0.10	0.10
Vitamin premix[1]	0.03	0.03	0.03
Mineral Premix[2]	0.10	0.10	0.10
Choline chloride 50%	0.10	0.10	0.10
Anti-coccidia	0.10	0.10	0.10
Total	100	100	100
Calculated Analysis			
ME, kcal/kg	2,900	2,830	2,800
Crude protein, %	22.30	20.00	18.70
Crude fat, %	4.42	3.964	4.659
Crude fiber, %	2.64	2.824	2.959
Calcium, %	0.87	0.89	0.79
Total phosphorus, %	0.75	0.78	0.75
Lysine, %	1.16	1.10	0.90
Methionine, %	0.50	0.43	0.38
Methionine + Cysteine, %	0.87	0.79	0.62

[1] Per kg: Vitamin A 55,000,000 IU; D3 12,500,000 IU; E 150g; K_3 10g; B_1 10 g; B_2 30g; B_6 20g; B_{12} 0.125g; Niacin 200g; Pantothenic acid 60 g; Biotin 0.75g; Folic Acid 5g. 2-Per kg: Iron 115,000mg, Manganese 50,000mg, Iodine 850mg, Selenium 150mg, Zinc 50,000mg, Copper 10,000mg, Carrier (+mg).

Methods

Metabolism trial was conducted for 6 days starting on day 23. Three broilers from each treatment were randomly selected and transferred to individual cages. Trays were installed under each cage to facilitate total collection of feces. On the 26th day of the feeding trial (4th day of the metabolism trial), feces were collected once daily for 3 consecutive days and were oven-dried at 70°C for another 3 days. The feces collected were pooled, weighed and ground to pass through a 0.5 mm screen and subjected to gross energy and crude protein determination in the laboratory. On the last day of the metabolism trial, the birds were slaughtered and the duodenum from each slaughtered bird was collected for histological examination.

The intestinal part collected was fixed in 10% formalin and submitted for histological examination to the Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, UPLB. Villi height and crypt depth were measured using Dino Capture 2.0 version 1.2.7. The data gathered were analyzed statistically following the analysis of variance (ANOVA) under a completely randomized design (CRD) using Tukey's HSD/B test to determine the difference between treatment means.

RESULTS AND DISCUSSION

Digestibility of nutrients in starter diets

Table 2 shows the coefficient of digestibility of the crude protein (CPCOD) and gross energy (GECOD) as well as the apparent metabolizable energy (AME) of the different treatments. The addition of 130 mg bLf/kg feed had the same digestibility and AME with the CTC-supplemented feed. Treatment 5, containing 520 mg bLf/kg feeds, had significantly lower CPCOD ($P<0.05$) than the rest of the treatments. Bovine Lf supplementation increased the GECOD of the starter diets but decreased upon increasing the inclusion to 520 mg/kg feed.The diet with 260 mg bLf/kg feed had the highest AME, which is comparable with the antibiotic group but decreased at increasing level of bLf (520 mg/kg feed).

Nutrient digestibility can be influenced by several factors such as the intestinal

Table 2. Coefficient of digestibility and apparent metabolizable energy of the starter diets.

COD	T1	T2	T3	T4	T5	CV
	Negative Control	1 g CTC 15%/kg feeds	130mg bLf/kg feeds	260mg bLf/kg feeds	520mg bLf/kg feeds	%
Crude Protein	70.10^{a}	75.54^{a}	72.59^{a}	71.87^{a}	62.15^{b}	4.01
GE	78.63^{c}	83.06^{ab}	82.35^{b}	84.25^{a}	78.23^{c}	1.15
AME (kcal/kg)	2578^{bc}	2776^{ab}	2647^{abc}	2819^{a}	2534^{c}	2.87

^{abc} Means within a row with different superscripts are different ($P<0.05$)

histology, particularly villus height and the crypt depth in the small intestine. With these, there is a potential of increasing the absorptive capacity of the small intestine (Han and Thaker, 2009). Moreover, bLf can inhibit bacteria that compete for nutrition with the host animal. Increased digestibility of nutrients leads to improved performance of the broilers (Rynsburger, 2009). Nutrients from the feeds can be converted into energy to promote growth. In effect, there will be an increase in the body weight and the broilers can have improved feed efficiency.

However, high inclusion of bLf (520mg/kg feeds) lowered the digestibility of nutrients in the feeds. Besides iron, Lf is capable of binding a large amount of other compounds and substances such as lipopolysacharides, heparin, glycosaminoglycans, DNA, or other metal ions like Al^{3+}, Mn^{3+}, Co^{3+}, Cu^{2+} and Zn^{2+} but its affinity for these other ions is much lower. Apart from CO_3^{2-}, Lf can bind other anions like oxalates and carboxylates. In this way, it is possible for Lf to affect the metabolism and distribution of various substances (Baker, 1994).

Table 3. Performance of broilers fed diets with different levels of bovine lactoferrin or chlortetracycline.

Parameter	T1	T2	T3	T4	T5	CV, %
	Negative Control	1 g CTC 15%/kg feeds	130 mg bLf/kg feeds	260 mg bLf/kg feeds	520 mg bLf/kg feeds	
Feed intake, g						
8-28d^{ns}	1740	1696	1711	1662	1691	2.78
2d-42d^{ns}	1982	1956	2000	1942	2001	3.39
8-42d^{ns}	3722	3651	3710	3604	3692	2.76
Body weight, g						
d7^{ns}	161	156	160	161	157	3.04
d28	1190^b	1220^a	1223^a	1243^a	1188^a	2.87
d42	1862^b	2078^a	2018^a	2103^a	2012^a	3.44
Body weight gain, g						
8-28d	1029^b	1063^a	1063^a	1081^a	1030^a	3.11
29-42d	672^b	858^a	795^a	861^a	824^a	8.13
8-42d	1701^b	1921^a	1858^a	1942^a	1854^a	3.69
Feed conversion ratio						
8-28d	1.69^b	1.60^{ab}	1.61^{ab}	1.54^a	1.64^{ab}	3.86
29-42d	2.99^b	2.28^a	2.52^a	2.27^a	2.45^a	9.32
8-42d	2.19^b	1.90^a	1.99^a	1.86^a	1.99^a	4.18
Livability, %	100	100	100	100	100	

^{abc} Means within a row with different superscripts are significantly different (P<0.05)

^{ns} Not significant (P>0.05)

Lactobacillus and Bifidobacterium are the two major beneficial genera of bacteria in the intestines of birds (Fooks and Gibson, 2002). These bacteria adhere to the intestinal epithelium of the host and can inhibit the growth of pathogenic bacteria such as *E. coli* and *Salmonella* that colonize the intestinal tract of chicken (Murry *et al.*, 2004). The mechanisms of inhibition of Lactobacillus include creating a low pH environment, competing for nutrients and binding sites with pathogenic bacteria (Raja *et al.*, 2009). They require iron under particular environmental conditions because iron has a role in the pyrimidine and purine metabolism of the bacteria (Elli *et al.*, 2000). Moreover, from the experiment conducted by Mac Leod and Snell (1947), it was found out that in all Lactobacilli species that were studied, manganese was essential for growth. In the present experiment, inclusion of bLf at 520 mg bLf/kg feed could have probably bound high amount of iron and manganese which are essential for growth of the beneficial bacteria. With this reason, population of the beneficial bacteria would decrease and this phenomenon can lead to the proliferation of the harmful bacteria such as *E. coli* and *Salmonella* as stated above which can inhabit the intestinal cells, causing atrophy of the villi and could compete for the nutrients that were supposed to be digested and metabolized by the broilers.

Performance parameters

Feed intake of the birds was not affected by bLf supplementation (Table 3). Since the dosage of CTC and bLf used was small, it was assumed that the palatability of the different treatment feeds were not affected, thereby resulting to same feed intake among treatments.

Bovine Lf supplementation increased the body weight of the broilers after consumption of the starter diet (P<0.05). The body weights of the birds that received different levels of bLf were comparable with the birds fed CTC. In effect, the body weight gain of the broilers in the starter stage and finisher stage also increased with the addition of bLf in the starter diet of the birds. At a level of 130 mg/kg feed, bLf was effective in improving the body weight and body weight gain of the broilers, which is comparable to the CTC group. The reason for this improved performance may be due to the action of bLf since it inhibits the growth of microbes present in the animal's digestive system which competes for the nutrients that should be available for the host animal (Wang *et al.*, 2006). *In vitro* study of Angeles *et al.* (2009) showed that carabao Lf (cLf) hydrolysate from the porcine pepsin had growth inhibitory effect against enteropathogenic *E. coli* and *S. typhimurium*. Moreover, if these organisms can be inhibited by bLf, the digestion of the feeds will be improved and the nutrients liberated will, thus, be converted into body weight. With the current experiment results, bLf supplementation can replace the use of in-feed antibiotics like CTC 15.00%.

At a level of 130 mg/kg feed, FCR was the same as the CTC-fed birds after consumption of starter feeds but bLf improved the FCR of the birds at 260 mg/kg feeds (P<0.05). This improvement may be due to the improvement of the gut environment and microbial flora brought about by the antimicrobial effect of bLf. Chavez *et al.* (2008) noted that Lf has activity against some gram-negative and gram-positive bacteria that are antibiotic resistant. With this, competition for nutrients by the different microorganisms present in the gut of the host and the host itself is prevented. Thus, nutrient can be used by the host animals leading to good performance.

FCR was also enhanced in weaned piglets fed diet with bLf in combination with other antimicrobial proteins as reported by Xiong *et al.* (2014). Geier *et al.* (2009) recorded feed efficiency of 1.69 and 1.70 for the two bLf treated groups against those treated with

Table 4. Comparison of the duodenal villi height and crypt depth of the broilers fed diets with different levels of bovine lactoferrin or chlortetracycline.

Parameter	T1 Negative control	T2 1 g CTC 15%/kg	T3 130 mg bLf/kg	T4 260 mg bLf/kg	T5 520 mg bLf/kg	CV, %
Villus height, um	673.11[d]	820.05[c]	1078.74[b]	1247.50[a]	926.56[c]	9.10
Crypt depth, um	411.97[b]	333.86[a]	279.55[a]	301.30[a]	335.24[a]	14.6

[abcde] Means within a row with different superscripts are significantly different (P<0.05).

zinc bacitracin and the control group for the broilers at day 32. On the other hand, other authors (Geier *et al*., 2009; Shan *et al*., 2007) suggested that there was no improvement in FCR with the use of bLf. There was no recorded mortality in this experiment for all the treatments; hence, the CTC used and the bLf (with small or large inclusions) did not affect the livability of the broilers. With the study of Sarelli *et al*., (2009) mortality of the challenged (infected) weaned pigs decreased as the level of Lf increased. There might be not enough defenses for the pigs with the low inclusion of Lf in comparison with those pigs given higher level of Lf.

With the performance of the birds, bLf could replace the use of CTC in the feeds of animals for human consumption since it was found out that Lf has no adverse effect on 12 adult humans with H. pylori infection (Opekun *et al*., 1999).

Histologic features of intestines

The average measurements of the duodenal villus height and crypt depth are shown in Table 4. Birds that received bLf on the diets had significantly longer villus height than the unsupplemented birds (P<0.05) even at low inclusion rate of 130 mg/kg feed. Addition of 260 mg bLf/kg feed resulted to longest villus height (P<0.05). Crypt depth of the duodenum were also improved by bLf at 130 mg/kg feed compared with the unsupplemented birds (P<0.05) and has the same effect as the CTC-treated feed.

According to Caruso *et al*. (2012), the basic functional unit of absorption in the intestine is the “crypt-villus”. Lf is well recognized for its antimicrobial property and also for promoting growth and cell differentiation. Several studies had the same result for the histological features of the intestines of the animals given Lf. The research of Caruso *et al*. (2012) on the histological study of the role of Lf in atrophy of the intestinal mucosa of rats resulted in an increase in the villus height and decrease in the crypt depth of the rats fed with Lf especially the group that fed on soy-based diet. This showed that Lf supplementation had an ability to stimulate the enterocyte production, affecting positively the weight of the animals. Lf increased the number and size of the pericrypt myofibroblasts and stimulated the restoration of atrophied villi for the rats given 200 mg/ kg/day. For the weaned pigs, there was an increase of 15.30% on the height of the villi and a decrease of 9.60% on crypt depth as reported by Wang *et al*. (2006).Their results showed that Lf could improve histological features of the small intestine and promote digestion and absorption of nutrients, regulate and enhance the immunity of neonatal pigs and, thereby, partially accounted for the improved growth during weaning. The structure of the intestinal mucosa

dictates gut health. Changes in intestinal histology such as shorter villus and deeper crypts have been associated with the presence of toxins (Xu *et al*., 2003). Antimicrobial peptides are gene-encoded natural antibiotics with potent and broad antimicrobial capabilities that function as first line of defense in the innate immunity of the host (Ganz, 2002; Lehrer and Ganz, 2002; Zasloff, 2002).

Similar to the result of this study, Humphrey *et al*. (2002) also recorded an increase in the villus height of the broilers fed a diet with rice expressing lysozyme and Lf as compared to the control. The height of the villi of the birds in their trial was 882 um on day 19 and was measured on the duodenum also. Increased villi length, and consequently surface area, would facilitate better nutrient digestion and absorption, explaining the improved efficiency of feed utilization. With the results, the birds performed similarly as the birds fed CTC and therefore, it can be suggested that bLf is as effective as CTC in promoting growth of the broilers. This supports the outcome of this experiment in which the longer the villus height, the greater is the body weight gain.

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