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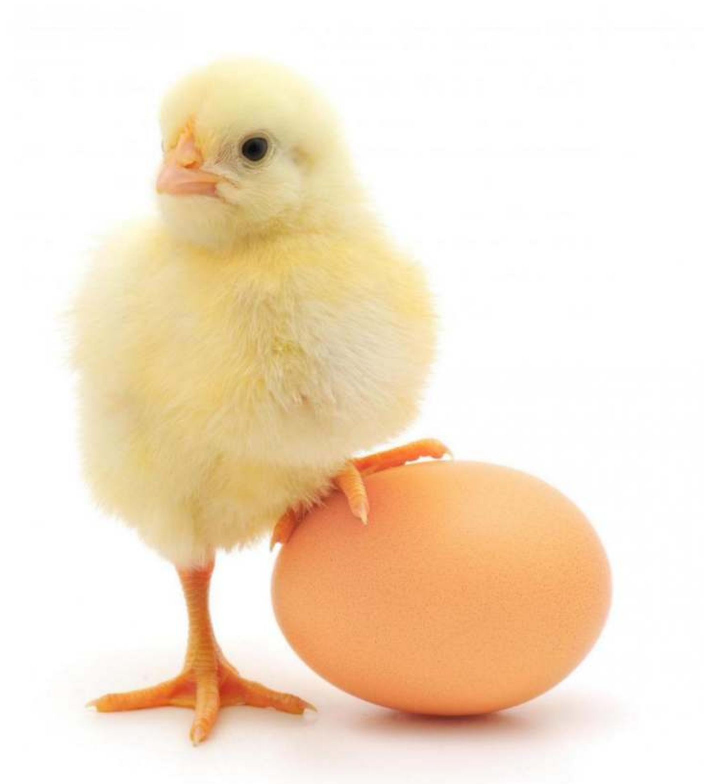

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Research Article
Effect of Fermented Cow and Soymilk as Probiotic on Energy Metabolism and Nutrient Retention in Broiler Chicken

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Abstract
Background and Objective: Probiotics have been reported to have a positive effect on gut morphology and absorption of nutrients in poultry birds. Therefore, the present study was carried out to evaluate the effect of fermented cow and soymilk on energy metabolism and nutrient retention of broiler chicken. **Materials and Methods:** A total of 100-day old chick were allocated to 4 treatment groups in a complete randomized design (CRD) and each treatment was replicated 4 times with 5 chicken in each replicate. The treatments consisted of basal feed T0 (control), T1 (basal feed+100% fermented cow milk), T2 (basal feed +75% fermented cow milk +25% fermented soy milk) and T3 (basal feed+50% fermented cow milk +50% fermented soy milk. **Results:** The results show that Apparent metabolizable energy (AME), Apparent metabolizable energy with nitrogen (AMEn), retention of dry matter (DM) and nitrogen are significantly ($p<0.05$) different in the groups if compared with control. The clearer effect is found in T2 (basal feed+75% fermented cow milk+25% fermented soy milk) and T3 (basal feed+50% fermented cow milk and 50% fermented soy milk). **Conclusion:** The administration of fermented cow milk and soy milk has a negative effect on retention of phosphorus and no effect on retention of calcium but it shows a positive effect on energy metabolism and retention of dry matter (DM) and nitrogen in broiler.

Key words: Broiler chicken, cow milk, nutrient retention, probiotic, soymilk

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Different kinds of feed additives such as antibiotics, probiotics and prebiotics are frequently supplemented in broiler feed for their better growth and survival[1]. However, the excessive use of animal growth promoter in animal diet enhances the development of antibiotic resistant bacterial strains, which transfer through the food chain and imposes negative effects on animal and human health[2]. As a result, the interest in the effects of probiotics on animal health and their performance has elevated.

Probiotic is a live microbe that have beneficial effects for health by improving the balance of microflora environment in the digestive tract[3]. Probiotics contain lactic acid bacteria that serves to improve the digestive and nutrient absorption processes[4]. Probiotics can increase the activity of enzymes such as sucrose, lactose and tripeptidase in the small intestines. Therefore the absorption of nutrients will be optimal in line with the increasing area of absorption. Then, probiotics can influence the intestinal anatomy like increased density and size of small intestinal villi[5].

Products containing probiotics are fermented milk. It can be produced from various types of milk such as soybean and cow milk. There is insufficient information available in the literature on the effect of fermented soy milk and cow milk on energy metabolism and nutrient retention in broiler chicken. Thus, the present study was undertaken to determine the effect of fermented cow and soymilk on energy metabolism and nutrient retention in broiler chicken.

MATERIALS AND METHODS

Animal: A total of 100 day old chicks were used in the study. Based on the similarity of body weight, all broilers were randomly assigned to 20 cages, 5 birds in each cage. There were 4 treatment groups and each treatment repeated 5 times. The feeding composition was based on the nutrient requirement for broiler strain Cobb (Table 1). The sample was reared in the cage of bamboo and had a size of 70×70×70 cm. Each cage was equipped with a husk base, round feeder and drinker. The nutrient and gross energy content of excreta were analyzed in the last week of study. Excreta were taken every day in the 6th week from each replicate. The parameters estimated were gross energy, dry matter, nitrogen, Calcium (Ca) and Phosphorus (P).

Processing of probiotics and treatments: Bacteria used for fermentation was *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium* sp.

Table1: Feed composition and ingredients of basal diet

Ingredients	Percentage
Yellow corn	60.00
Soya bean meal	31.05
Fish meal	4.20
Vegetable oil	2.26
Bone meal	1.28
$CaCO_3$	0.50
Premix	0.50
Lysin	0.06
Methionine	0.15
Calculated values	
ME (kcal kg^{-1})	3011.00
CP (%)	21.97
Ca (%)	1.10
P (%)	0.45
Methionine (%)	0.58
Lysin (%)	1.33

Probiotics were prepared at the Research and Testing Laboratory of the Faculty of Animal Husbandry of Padjadjaran University. The following steps were used:

- The four-strain probiotic product was cultured in MRS in de Man, Rogosa, Sharpe bouillon (MRS) Broth medium and then incubated at 37°C for 16 h to obtain the first derivative of pure derivative (F1)
- F1 was taken as much as 0.1% (5µ) and each was cultured in de Man, Rogosa, Sharpe bouillon (MRS) Broth medium and then incubated at 37°C for 16 h to obtain a second derivative of pure F2
- F2 was taken as much as 0.1% (5 µ) and each was cultured in sterile skimmed milk media and then incubated at 37°C for 16 h to obtain a pure culture on milk medium
- Breed pure in milk medium was taken as much as 5% and each cultured in sterile skimmed milk media and then incubated at 37°C for 16 h to get ready to use
- Fermented cow and soy milk was sterilized and mixed in one container with a predetermined ratio according to the treatments used in the study, then each was included as 1.25% and incubated at 37°C for 16 h

The four combination treatments were prepared from fermented cow and soy milk. The doses, which is added to the broiler basal diet, was 1.25% based on Adriani *et al*.[6].

Treatments were:

T0 = Without probiotics
T1 = 100% fermented soy milk
T2 = 75% fermented cow milk+25% fermented soy milk
T3 = 50% fermented cow milk+50% fermented soy milk

Table 2: Energy retention and nutrient metabolism values in boiler chicken

Parameters	T0 control (without any probiotic)	T1 (100% fermented cow milk)	T2 (75% fermented cow milk and 25% fermented soy milk)	T3 (50% fermented cow milk and 50% Fermented soy milk)
AME (kcal kg^{-1})	2946.00 ± 64^{a}	2863.00 ± 48^{b}	3076.00 ± 48^{c}	3131.00 ± 32^{c}
AMEn (kcal kg^{-1})	2922.00 ± 64^{a}	2839.00 ± 48^{b}	3051.00 ± 48^{c}	3106.00 ± 32^{c}
Retention of Ca (%)	33.00 ± 11.99^{a}	20.55 ± 8.03^{a}	26.46 ± 5.88^{a}	26.62 ± 8.64^{a}
Retention of P (%)	53.64 ± 2.77^{a}	48.75 ± 5.22^{a}	0.03 ± 10.35^{b}	4.05 ± 7.03^{b}
Retention of DM (%)	73.09 ± 2.26^{a}	70.59 ± 1.71^{a}	75.88 ± 1.99^{b}	76.29 ± 0.74^{b}
Retention of N (%)	81.24 ± 3.05^{a}	82.55 ± 0.73^{a}	86.26 ± 0.73^{b}	86.80 ± 1.40^{b}

Parameters estimated

Energy metabolism: Apparent metabolizable energy (AME) and Apparent metabolizable energy with nitrogen correction (AMEn) were estimated by using the ratio of dry matter intake to output through the use of an internal indicator, such as lignin:

$$\text{AME} = \text{GE(r)} - \left[\text{GE(e)} \times \left(\frac{\%\text{lignin (r)}}{\%\text{lignin (e)}}\right)\right]$$

$$\text{AMEn} = \text{GE (r)} - \left[\text{GE (e)} \times \left(\frac{\%\text{lignin (r)}}{\%\text{lignin (e)}}\right)\right] - \left\{\text{K} \times \left[\text{N(r)} - \left[\text{N(e)} \times \left(\frac{\%\text{lignin (r)}}{\%\text{lignin (e)}}\right)\right]\right]\right\}$$

Nutritient retention: Nutrient retention was the assumption value of dry ingested ingredients that was the difference between the number of nutrients consumed and the number of nutrients in the excreta. Nutrient retention was determined by using the ratio of dry matter intake to output through the use of an internal indicator, such as lignin. Calculation of retention was done by using various equations and retention of dry matter, nitrogen, calcium and phosphorus.

$$\%\text{Nutrient retention} = 100\% - 100 \times \left(\frac{\%\text{lignin in the diets}}{\%\text{lignin in the excreta}} \times \frac{\%\text{Nutrient in excreta}}{\%\text{Nutrient in the diets}}\right)$$

Statistical analysis: The statistical analysis was performed to know the effect of treatment using one-way analysis of variance (ANOVA). Results obtained were presented as mean±standard error. The significant differences among different treatment means were investigated using Duncan's multiple range test[7] by considering differences significant at $p<0.05$.

RESULTS AND DISCUSSION

The result showed that AME and AMEn were significantly ($p<0.05$) different in the group where probiotic in the cow and soymilk was used to be compared with control. The highest value of AME (3131±32 kcal kg^{-1}) and AMEn (3106±32 kcal kg^{-1}) were found in T3 (50% Fermented cow milk and 50% fermented soy milk). The lowest value of AME (2863±48 kcal kg^{-1}) and AMEn (2839±48 kcal kg^{-1}) were observed in T1 (100% fermented cow milk). However, retention of Calcium (Ca) and Phosphorus (P) decreased if compared with control (T0). The clearer effect was observed on the retention of phosphorus, which declined significantly ($p<0.05$) in the use of fermented cow milk and soymilk. The highest retention of Ca (33.00±11.99) and P (53.64±2.77) was observed in the control group (T0). This indicates that utilization of probiotics in fermented cow and soymilk has a negative effect on retention of calcium and phosphorus in broiler.

The retention of dry matter (DM) and nitrogen (N) was significantly ($p<0.05$) different in the treatment group if compared with control. The highest value of retention of DM (76.29±0.74) and N (86.80±1.40)was found in the T1 (100% Fermented Cow Milk). The results of the present study are in line with Palliyaguru *et al.*[8], who observed the improvement in nutrient retention in broilers, which were supplemented by probiotic. This effect may be mediated by an improvement in the main functions of digestion, absorption and propulsion in the gastrointestinal tract[9].

Bifidobacteria have a positive interaction with carbohydrates in fermented soy milk. Like most of the intestinal bacteria, Bifidobacteria are saccharolytic and suspected to play an important role in carbohydrate fermentation in the colon. Bifidobacteria can utilize a different range of dietary carbohydrates that escape degradation in the upper parts of the intestine, many of which are plant-derived oligo- and polysaccharides. The physiological data has confirmed that Bifidobacteria can ferment various complex carbons such as gastric mucin, xylooligosaccharides, soybean oligosaccharides, malto-oligosaccharides, fructo-oligosaccharides and other plants although the ability to metabolize particular carbohydrate depends on strains[10,11]. The genome of *Bifidobacteria* shows the adaptation of the metabolic complex of carbohydrate in the gastrointestinal tract. The indigestible saccharide is useful to increase the number of intestinal *Bifidobacteria*[12]. Lactulose, one of the indigestible saccharides, provides as a *Bifidus facto*[13]. Raffinose is more effective than lactulose in promoting *Bifidobacterial* growth[14]. The fraction of stachyose and raffinose of soybean significantly increase the number of *Bifidobacteria*[15].

Generally, gut bacteria can degrade the polymeric carbohydrates being the low molecular weight of oligosaccharides, which can be degraded to monosaccharides by using a wide range of enzymes. In the case of *Bifidobacteria*, these monosaccharides are converted to intermediates of the hexose fermentation pathway, also called fructose-6-phosphate shunt or 'bifid' shunt and ultimately converted to short-chain fatty acids and other organic compounds, some of which have a benefit to the host. Short-chain fatty acids can stimulate the absorption of sodium and water in the colon and is known for its ability to induce enzymes, which will promote the mucosal restitution[16].

Probiotic-based administration multi-strain of lactic acid bacteria and *Bifidobacteria* spp. can increase the number of goblet cells and the length of Villus[17]. The higher synthesis of the mucin gene after probiotic administration may positively affect bacterial interactions in the intestinal digestive tract, intestinal mucosal cell proliferation and consequently efficient nutrient absorption.

Phosphorus retention in chickens in T2 (50% fermented cow milk and 50% fermented soybean milk) and T3 (75% fermented cow milk and 25% soy milk) is significantly lower than T0 (Control) and T1 (100% fermented cow milk). As discussed earlier, short-chain fatty acids is an acidic organic compound. The phosphorus status setting in the body is regulated by the urinary excretion mechanism. Acid conditions can increase renal excretion and this causes a decrease in plasma phosphorus levels.

CONCLUSION

In conclusion, the feeding of cow and soymilk based probiotics a had negative effect on retention of calcium and phosphorus but showed a positive effect on energy metabolism and retention of dry matter and nitrogen in broiler chicken.

SIGNIFICANT STATEMENT

This study has discovered that the administration of fermented cow milk and soy milk in the diet of broiler is beneficial in improving the energy of metabolism and retention of dry matter and nitrogen. This study will help the researchers to explore the effect of various combinations of fermented cow milk and soy milk in improving the gut digestive and absorptive processes.

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