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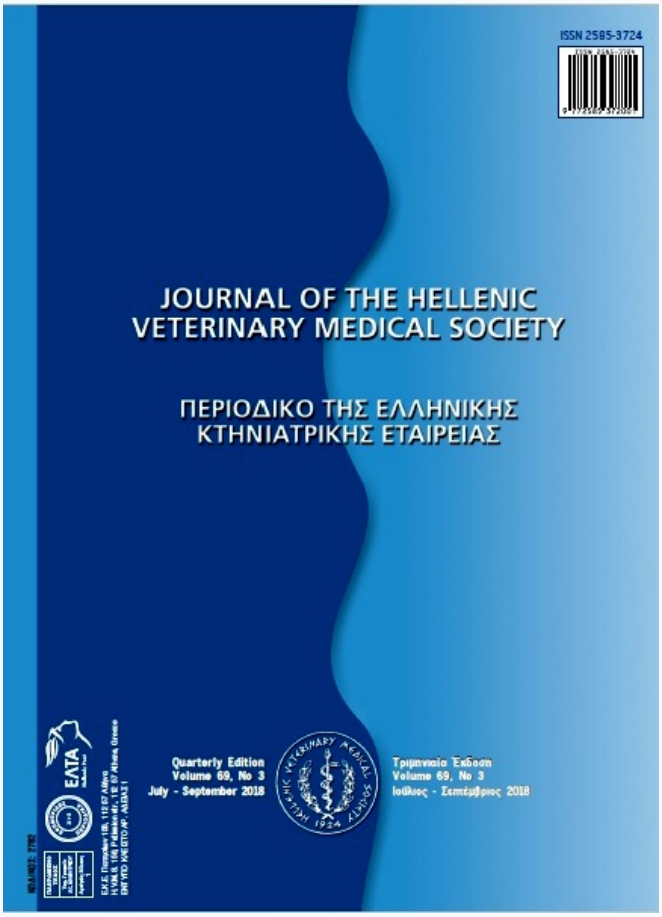

Effect of microencapsulated butyric acid supplementation on growth performance, ileal digestibility of protein, duodenal morphology and immunity in broilers

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Research article
Ερευνητικό άρθρο

Effect of microencapsulated butyric acid supplementation on growth performance, ileal digestibility of protein, duodenal morphology and immunity in broilers

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ABSTRACT. This experiment was conducted to evaluate the effect of microencapsulated butyric (MEB) acid on growth performance, apparent ileal digestibility of protein (AID), duodenal morphology and immunity in broilers reared to 35-days. In total, 336 one-day-old Hubbard classic broiler chicks were randomly assigned to 4 dietary treatments (Control, 0.25, 0.35 and 0.45g/kg of MEB). Each treatment was replicated 3 times with 28 birds in each replicate. Feed intake, body weight gain and feed conversion ratio (FCR), parameters of growth performance and intestinal morphology, AID of protein and immunity parameters were evaluated. At the end of the experiment (35-d), 3 birds / replicate were randomly selected and slaughtered to collect blood, duodenal samples, and ileal digesta. The result indicated improved body weight gain ($P<0.05$), feed conversion ratio ($P<0.05$) and AID ($P<0.05$) whereas, treatments remained unresponsive with respect to feed intake ($P>0.05$), duodenal villous height ($P>0.05$) and antibody titer against Newcastle disease (ND) ($P>0.05$). There is an indication that MEB improves the digestion and consequently bird's performance.

Keywords: Butyric acid, duodenal morphology, immunity, broilers

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INTRODUCTION

Better intestinal health and high digestibility of nutrients in broilers are supremely important in order to achieve higher body weight and better feed conversion ratio (FCR) (Roberts et al., 2015). Maintenance of gut development and health is very important to support development and health of the bird (Choct et al., 2009).

Organic acids (OA) and their salts are generally considered as harmless and have been approved by most technologically advanced countries to be used as a feed additive for animals. The acidifiers, including sodium butyrate (SB) is known for decreasing the gut mucosal pH, thus creating an acidic environment for the growth of normal commensals. OA are one of the potent and effective feed additives that can be used in animal nutrition to achieve higher body weight (BW) and better feed conversion ratio (FCR) (Abdel-Fattah et al., 2008). Among the OA supplements, especially BA is a suitable candidate to improve gut health resulting in more nutrients being absorbed throughout the gastrointestinal tract (GIT). Butyric acid ($CH_3CH_2CH_2$-COOH), which is a short chain fatty aicd (SCFA) having 4 carbons. Butyric Acid improves bioavailability which helps enterocytes to absorb more nutrients for the development of birds. The BA is a readily available energy source for intestinal villi and stimulates their differentiation and multiplication (Dalmasso et al., 2008) and consequently increased feed efficiency (Adil et al., 2011). It induces the production of host defense peptides when it enters in the blood stream (Guilloteau et al., 2009). These peptides stimulate the repair and development of the lower intestinal tract by improving cell proliferation (Bartholome et al., 2004). Butyrate produced by fermentation of carbohydrates is rapidly absorbed and locally affect the large intestine. Endogenous butyrate imparts no direct useful effects in small intestines (Niewold, 2014), however, the exogenous uncoated BA is readily absorbed and metabolized by crop and proventriculus of birds before reaching the small intestine (Borne et al., 2015; Kaczmarek et al., 2016).

It has been observed that the microencapsulated (coated with fatty acid matrix) type of organic acid was more effective than an antibiotic growth promoter (Enramycin) in rising growth performance in broilers. The microencapsulated butyrate delivered portion of the butyrate to be free further distal in the intestinal tract because of slow release during digestion and causes mucosal modulation in the gut. Its use led to a tendency towards better growth performance, lower colonization and fecal shedding of *Salmonella* compared to the non-protected feed supplements (Chamba et al., 2014). Consequently, the protection of BA with microencapsulation, such as MicroPEARL® technology (Kemin, Herentals, Belgium), improves its efficacy. The MicroPEARL technology helps to prevent the rapid absorption of BA in the GIT, and thus its utilization, thereby increasing the surface area exposed to the molecule (Smith et al., 2012).

To-date, limited reports have been published to evaluate the effects of SB on performance of broilers, ileal digestibility of protein, duodenal morphology and immunity in broilers. A comprehensive study was, therefore, needed to assess such effects in commercial chickens. Keeping in view the above-mentioned properties of BA, in this experiment, MicroPEARL encapsulation using hydrogenated palm oil calcium butyrate (MEB) (ButiPEARL, Kemin) was used in broilers to study its effect on overall performance of broilers, ileal digestibility of protein, duodenal morphology and immunity in broilers.

MATERIALS AND METHODS

The study was carried out in accordance with the guidelines of Animal Care and ethics Committee, University of Veterinary and Animal Sciences, Lahore, Pakistan. The trial was conducted at Research and Development Farm Sharif Feed Mills (Pvt) Ltd, Okara, Pakistan for the duration of 35 days. In total, 336 one-day-old broiler chicks were procured from a local commercial hatchery and randomly assigned to 4 dietary treatments as MEB-I (control), MEB-II (0.25g/kg), MEB-III (0.35g/kg) and MEB-IV (0.45g/kg) of MEB in control diet. The MEB (ButiPEARL™, Kemin) contained 50% calcium butyrate. Each treatment was replicated thrice with 28 birds each. Experimental birds had been raised in 12-floor pens on a concrete floor with rice husk as a bedding material. All standard management practices were followed throughout the trial. Birds

were vaccinated according to the prescribed schedule. Birds were observed twice daily for any clinical sign.

Table 1 shows the formulation and nutrient composition of the control diets which was formulated to meet or exceed the nutrient requirements of broiler chickens (NRC 1994). All the diets were fed in crumbs form in two feeding phase starter (1 to 21 days) and grower (22 to 35 days) and all diets were iso-caloric & iso-nitrogenous in both phases. In all diets Celite® at 2% were added on 32-day of the experiment as an inert marker for the estimation of AID of protein.

Table 1. Composition of the diets (% as-fed basis).

Ingredients	Starter	Grower
Maize	42.3	52.5
Rice Tips	15	5
Rice Polish	6	2.95
Soybean	19.75	18.15
Rape Seed Meal	_	2
Canola Meal	11.15	12
Fish Meal	1.5	1.5
Animal protein concentrate	1.5	2
L-Lysine HCl	0.62	0.55
DL-Methionine	0.17	0.15
L-Threonine	0.09	0.09
DCP	0.73	0.55
Calcium Carbonate	0.57	0.57
Sodium Bicarbonate	0.13	0.1
Sodium Chloride	0.42	0.25
**Vitamin mineral premix	0.07	0.09
Tallow	_	1.60
Phyzyme XPTPT	0.01	0.01
Analyzed Nutrients (%)		
ME kcal/kg	3070	3127
CP (%)	21.67	20.73
dLys (%)	1.20	1.10
dM+C (%)	0.90	0.85
CF (%)	4.32	4.97
Calcium (%)	0.95	0.87
Avail. P (%)	0.56	0.44

**Premix composition (per kg of diet): retinol12000 IE, cholecalciferol 2400 IE, dl-a-tocopherol 0.05g, thiamine 2.0 mg, riboflavin 7.5 mg, pyridoxine 3.5 mg, cyanocobalamin 20 mcg, niacin 35 mg, D-pantothenic acid 12 mg, choline chloride 460 mg, folic acid 1.0 mg, biotin 0.2 mg, iron 80 mg, copper 12 mg, manganese 85 mg, zinc 60 mg, cobalt 0.40 mg, iodine 0.8 mg,selenium0.1 mg, anti-oxidant mixture125 mg.
dLys= digestable Lysine, ME=metabolizable energy, CP=crude protein, dM+C= digestible Methionine + Cysteine , CF= crude fiber

The birds had *ad libitum* access to water and feed. Body weight and feed intake (FI) were measured weekly with the pen as the experimental unit. Before weighing, mean body weight gain, FI, and FCR ratio were used to determine the growth performance.

At the end of the experiment, n=3 birds/replicate were selected randomly for the collection of ileal digesta and blood samples. To harvest serum, blood samples were allowed to stand at room temperature for 1 hour and then centrifuged (Beckman J25I; Beckman Instruments, Inc. USA) at 1500 × g at 4°C for 20 min. The serum was divided into aliquots and stored at -20°C for analysis of antibody titer against Newcastle disease (ND). The stored serum samples were used to determine the antibody titer against NDV through Haemagglutination and Haemagglutination-inhibition (HI) tests. Digesta samples within a pen were pooled and stored at -20°C until used for acid insoluble ash (AIA) and CP analysis (Lemme et al., 2004). Feed and digesta samples were analyzed for crude protein and AIA contents using a standard method (AOAC International, 2000).

The data collected for AIA and CP contents of the feed and digesta samples were used to calculate the ileal digestibility of proteins using following equation (Ravindran et al., 2006).

Apparent Protein Digestibility (%) = [(NT/AIA) d − (NT/AIA) I] / [(NT/AIA) d] x 100

Where, (NT/AIA) d = ratio of nutrient and AIA in diet, and (NT/AIA) I = ratio of nutrient and AIA in ileal digesta.

On slaughtering, thymus, spleen and bursa were collected and weighed. The weight of these organs g/100g of live body weight were calculated (Taherpour and Ghasemi, 2014).

Histomorphological Evaluation

Gut morphology is an important indicator of gut health. Histomorphological evaluation included

determination of the villus height (VH), crypt depth (CD) and villus height to crypt depth ratio (VH:CD). Duodenal samples from slaughtered birds were collected as described by (Qaisrani et al., 2015). Briefly, a duodenal sample, 2 cm in length was collected from the middle of the duodenum, washed with normal saline (0.9% NaCl) solution and instantly stored in 10% formalin solution until further processing. The preserved duodenal tissue samples were processed according to conventional haematoxylin and eosin method described by (Chen et al., 2016). The tissue slides were examined for villus height and crypt depth using a compound microscope (Olympus CX31, Olympus USA) equipped with a digital imaging system (Olympus DP20, Olympus USA).

Statistical Data analysis

The collected data were analyzed through completely randomized design (CRD) under one way analysis of variance (ANOVA) (Steel et al., 1997). Data were mentioned as means ±SEM (standard error of the mean) and analyzed using GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). Means were separated through Duncan's Multiple Range test using SAS 9.1. Differences were taken as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Table 2 shows the effect of MEB supplementation on FI, BWG and FCR. Feed intake ($P > 0.05$) was unaffected by supplementation of different levels of MEB. However, it was numerically lower in birds fed 0.35g/kg MEB. The supplementation of encapsulated butyric acid positively influenced the BWG ($P < 0.05$) and FCR ($P < 0.05$) but the results were more pronounced with 0.45g/kg of MEB. The present findings are in coherence with Levy et al. (2015) and Kaczmarek et al. (2016) who reported that graded levels of MEB supplementation in broiler diet improved broilers performance without affecting FI. The results of this experiment are also in line with the reports of other researchers (Chamba et al., 2014; Eshak et al., 2016), who reported that addition of BA in broilers diets improved BWG. The improvement in BWG and FCR may be due to microencapsulation of butyric acid with palm oil allowed for the target release of butyrate at the ileum level, improvement in duodenal morphology and especially improved protein digestibility.

In contrary to current findings Mahdavi and Torki, (2009) and Aghazadeh and TahaYazdi, (2012) reported that different levels of unprotected butyric acid did not influence the BWG and FCR. This might be due to the fact that BA was unprotected, which was absorbed in gizzard and proventriculus and did not reach the target site. Smith et al., (2012) revealed that encapsulating butyrate delays BA absorption, allowing it to reach the small intestine.

Small intestine is the site for absorption in which the available nutrients are taken up through epithe-

Table 2..- Effect of microencapsulated butyric acid on feed intake, body weight gain and feed conversion ratio at 35-d (Mean ± SEM).

Groups	Feed intake (g)	Body weight gain (g)	Feed conversion ratio (g/g)
MEB-I	3190.84±55.63	1823.50^{b}±34.24	1.75^{b}±0.01
MEB-II	3179.97±69.93	1937.32^{a}±23.71	1.64^{a}±0.02
MEB-III	3177.22±44.83	1940.02^{a}±14.45	1.63^{a}±0.01
MEB –IV	3153.31±48.57	1967.5^{a}±30.56	1.60^{a}±0.02
P value	0.9685	0.0222	0.0056

$^{a-b}$Means with different superscripts in a column are significantly different (P<0.05)

Table 3.. Effect of microencapsulated butyric acid on duodenal villus height (VH), crypt depth (CD), villus height to crypt depths ratio(µm) and apparent ileal digestibility (AID) of protein at 35-d (Mean ± SEM).

Groups	Villus height(µm)	Crypt depth(µm)	Villus height to crypt depth ratio (µm)	Apparent ileal digestibility of protein (AID %)
MEB-I	1043.71±22.29	103.33±8.81	10.21±0.71	70.87^{b}±1.41
MEB-II	1223.79±53.70	145.33±3.92	8.42±0.34	73.30^{b}±0.35
MEB-III	1323.33±98.20	147.33±23.91	9.26±1.01	$74.66^{a,b}$±0.66
MEB-IV	1373.33±89.87	130.00±5.13	10.54±0.35	76.72^{a}±0.76
P value	0.0501	0.1448	0.1813	0.0098

$^{a-b}$Means with different superscripts in a column are significantly different (P<0.05)

lial cells and drained into the general circulation. Architectural modulation of the small intestine is assumed to have a relationship with production performance of animals (Table 3). Butyrate acts as a rich source of energy for the enterocytes (Ahsan et al., 2016), and it may possibly increase the cell mitosis in the crypts. The SB may protect the mucosal epithelium from injury and alleviate the enteropathic stress (Ashraf et al., 2013) by increasing thyroid hormone in the circulation. We found improved histomorphometrics in MEB offered groups in duodenum and jejunum. These findings proposed that the incoming ingesta containing MEB at ileum had earlier been presented to utmost absorption in the former gut lumen and displayed better effect there. Duodenum is the major site of digestion in broilers. Butyrate supplementation, however, did not significantly influence villus height (P > 0.05), crypt depth (P >0.05) and villus to crypt depth ratio (P >0.05) of duodenum (Table 3). Our findings are in line with Levy et al. (2015), who did not find any significant effect on duodenal morphology with the addition of MEB. Likewise, Smulikowska et al. (2009) reported non-significant effect of coated BA supplementation on jejunal morphologyon.

In contrast to our findings, Kaczmarek et al. (2016) found that microencapsulation made a difference and the supplementation of MEB had a significant effect on VH. Similarly, morphometric results, Panda et al. (2009) who reported that BA, regardless of concentrations in feed, increased VH. This can be attributed to the BA that is a readily available energy source for intestinal villi and stimulates their differentiation and multiplication (Dalmasso et al., 2008). These contrary results of duodenal morphology might be due to day on which samples were taken or dose difference.

AID of protein (P<0.05) was higher with a higher level of MEB supplementation (Table 3). The results of AID are in line with Kaczmarek et al. (2016) who reported that encapsulated calcium butyrate supplementation improved ileal digestibility of amino acid in broilers. Likewise, Jahanian and Golshadi, (2015) found that butyric acid glycerides (BAG) improved ileal protein digestibility in laying hens. Our results are also in agreement with Dehghani-Tafti and Jahanian, (2016). The improvement in AID might be due to the fact that butyric acid supplementation increased pancreatic fluid, amylase, and dose dependent secretion of trypsin (Ohbo et al., 1996; Sileikiene et al., 2005). Proteolysis of proteins by pepsin produced peptides which activated the release of hormones including cholecystokinin and gastrin (Adil et al., 2011).

It had been reported that BA and it glycerides improved immunity in broilers. In this experiment no significant difference was found on the weight of immune organs and antibody titer of NDV at 35th day of age (Table 4). Mahdavi and Torki, (2009) found that inclusion of BAG in broilers diet did not

Table 4. Effect of microencapsulated butyric acid on relative organs weight* and antibody titer against Newcastle Disease at 35-d (Mean ± SEM)

Groups	Spleen*	Thymus*	Bursa of Fabricius*	ND titer
MEB-I	0.096±0.010	0.108±0.008	0.095±0.006	6.11±0.26
MEB-II	0.093±0.006	0.133±0.010	0.128±0.015	6.23±0.35
MEB-III	0.098±0.006	0.112±0.005	0.118±0.009	7.00±0.23
MEB-IV	0.104±0.005	0.112±0.010	0.113±0.015	6.33±0.28
P value	0.7884	0.2030	0.3100	0.1142

*Relative organs weight = organ weight/body weight×100.

have significant effect on spleen, thymus and bursa of Fabricius weight at an age of 35 days. Contrary to our findings, Jahanian (2011) reported that the supplementation of 0.2% BAG improved ND antibody titer at the 12th day post vaccination. Eshak et al. (2016) also reported contrary findings to our findings, which might be due to the longer interval among sampling and vaccination days.

CONCLUSION

It can be concluded from the present study that microencapsulated butyric acid supplementation at the levels of 0.25g/kg to 0.45g/kg in broilers diet improve body weight gain and feed conversion ratio and protein digestibility. It did not influence duodenal morphology in the broilers.

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FINANCIAL DISCLOSURE STATEMENT

This study was not funded by any funding agency and was conducted at the expense of the research group to combat the rising problem in the poultry industry of the country.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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