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STUDIES ON THE NUTRIENTS METABOLIZABILITY FOR THE EFFECT OF DIETARY SUPPLEMENTATION OF SALTS OF DIFFERENT LEVELS OF ORGANIC ACIDS MIXTURE OF LAYING HENS

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ABSTRACT
The present study was carried out to determine the effect of dietary organic acids mixture supplementation on Nutrients metabolizability. A total 24 week old 140 white leghorn laying hens which were randomly distributed to seven dietary treatment groups, each containing 20 hens. The hens were fed (18% CP% and 2697 Kcal KgG[1] ME) *i.e.* Supplemented with T_1 (0% control), T_2 (0.5% sodium-butyrate), T_3 (1.0% sodium-butyrate), T_4 (1.5% sodium-butyrate), T_5 (0.5% calcium-propionate), T_6 (1.0% calcium-propionate) and T_7 (1.5% calcium-propionate). The value of nitrogen corrected ME (kcal/kg) and gross energy metabolizability (%) were significantly ($P<0.05$) higher in treatment T_3 (1.0% sodium-butyrate) and T_6 (1.0% calcium-propionate) as compared to all other treatments. The nitrogen corrected ME (kcal/kg) and gross energy metabolizability (%) were significantly ($P<0.05$) higher in T_3 (1.0% sodium-butyrate) and T_6 (1.0% calcium-propionate) as compared to T_1 (control), T_2 (0.5% sodium-butyrate), T_4 (1.5% sodium-butyrate), T_5 (0.5% calcium-propionate) and T_7 (1.5% calcium-propionate) groups. In nutshell, energy metabolizability was significantly ($P<0.05$) improved by supplementation of salts of organic acids in the ration of layers. Supplementation of salts of organic acids significantly improved nutrients metabolizability, energy metabolizability.

KEYWORDS: Calcium-propionate dietary organic acids, Dietary treatments, Egg quality, Laying hens and Sodium butyrate.

INTRODUCTION
Antibiotics have been widely used in poultry production for decades to improve growth rate and feed conversion efficiency, however, their use as growth promoters in the poultry industry has been intensively controversial because of the development of bacterial resistance and potential consequences on the human health (Ratchliff, 2000). In response to this apparent threat, the European commission (EC) decided to phase out, and ultimately ban (January 1st 2006), the marketing and use of antibiotics as growth promoters in feed (EC Regulation No. 1831/2003). Organic acids and their salts are generally regarded as safe and have been approved by most member states of European Union (EU) to be used as feed additives in the animal production (EFSA (2011). The advantage of salts over acids is that they are generally odourless and easier to handle in the feed manufacturing process owing to their solid and less volatile form Huyghebaert *et al.* (2011). Organic acids can serve as a meaningful tool to controlling all enteric non-pathogenic and pathogenic especially acid-intolerant bacteria like *Escherichia coli, salmonella* and *campylobacter* species [3]. Non-antibiotic alternatives to antibiotic growth promoters have been proposed for use in animal diets due to concerns about the safety in both animals and humans. Metabolizable energy requirements of commercial layers depend on environmental temperature (Rostagno *et al*., 2005; Sakomura *et al*. 2005); it increases when the environment is cold or hot (Sakomura *et al*., 2005). Therefore, under heat stress situations, increasing energy levels in the diet of commercial layers by the inclusion of oil may compensate the low feed intake and supply the higher energy requirements. Nevertheless, Usayran *et al.* (2001) did not find any relationship between environmental temperature and dietary oil levels fed to commercial layers. Because protein presents the higher heat increment among nutrients (Pond *et al*., 2005), it is usually recommended to reduce dietary protein levels when poultry are reared in hot environments. Nevertheless, recent studies with broilers have shown that it is better to increase dietary CP level to compensate the low protein intake resulting from exposure to heat (Faria Filho, 2006). No studies on this matter were found with commercial layers in literature. The aim of this study was to investigate the effects of organic acid mixture at different levels of supplementation in the diet of laying hens on Nutrients metabolizability and serum parameters of laying hens.

MATERIALS & METHODS
Experiment and data structure
All the experimental procedures have been conducted in accordance with the guidelines laid down by the Institutional Ethics Committee. The investigation was conducted at poultry farm, Department of Animal Genetics and Breeding, College of Veterinary sciences, LUVAS, Hisar for the year 2016. For this study one hundred and forty single comb white leghorn laying hens at 24 weeks of age were randomly distributed to seven

dietary treatment groups *i.e*. T_1 (control), T_2 (0.5% sodium-butyrate), T_3 (1.0% sodium-butyrate), T_4 (1.5% sodium-butyrate), T_5 (0.5% calcium-propionate), T_6 (1.0% calcium-propionate) and T_7 (1.5% calcium-propionate), consisting of five replications of four birds each in each treatment. Based upon the proximate composition and metabolizable energy of feed ingredients the layer's control ration having maize grain as energy source was formulated as per BIS (2007). All the diets were analysed for proximate principles (AOAC, 2007) and were randomly divided into 7 groups in Completely Randomized Design (CRD). The hens were housed individually in cages. All the diets were prepared to be isocaloric and nitrogenous. They were reared under identical conditions of environment and management of light, water, disease control *etc*. Feed and water were supplied ad lib. The different dietary treatments were, as given below:- T_1, Basal diet (Control) as per BIS, 2007 Standard; T_2, Basal diet + Sodium butyrate @ 0.5%; T_3, Basal diet + Sodium butyrate @ 1.0%; T_4, Basal diet + Sodium butyrate @ 1.5%; T_5, Basal diet + Calcium propionate @ 0.5%; T_6, Basal diet + Calcium propionate @ 1.0% and T_7, Basal diet + Calcium propionate @ 1.5%. Feed additives and supplements were premixed and then mixed with weighed quantity of feed ingredients to make a homogenous mixture of rations. The cost of different experimental diets T_1 (control), T_2, T_3, T_4, T_5, T_6 and T_7 were Rs. 22.23, 22.68, 23.13, 23.58, 22.65, 23.08 and 23.50/kg, respectively.

TABLE 1: The ingredients and chemical composition of control diet

Ingredient composition	
Ingredients	(Kg/100kg feed)
Maize	50
Soybean meal	13
Groundnut cake	7
DORP	12
Rice Polish	5
Fish Meal	6
Mineral Mixture	3
Salt	1
Shell Grit	3
Total	100

* Calculated

Feed additives included Spectromix-10g and Spectromix-BE-10g per 100kg feed

The study was undertaken from 24 to 40 weeks of age of layers in first phase of production cycle. The entire duration of study was divided into eight periods of 14 days each. A metabolism trial was conducted at the end of the experiment for each treatment for nutrient retention and energy metabolizability. Five birds from each treatment were randomly selected and transferred to metabolic cages. A collection period of five days was provided for collection of faeces samples.

Collection of Feed and Excreta Samples

Weighed polythene sheets of appropriate size were spread over the faecal tray for the collection of mixed excreta daily at 10:00 A.M. Weighed quantity of feed was offered at the same time to all the birds in the cages. The excreta dropped on the sheets were weighed along with polythene sheets and weight of excreta in each case was calculated by difference. New polythene sheets of same size were weighed and spread on the trays to repeat observations for the next day. The excreta on each polythene sheets were thoroughly mixed and homogenous samples were taken for analysis of excreta in plastic bottles and were kept in deep freeze. On the last day of collection, the excreta samples were kept at room temperature. The samples were taken to determine moisture and nitrogen contents. The dried samples were kept for energy estimation.

Feed offered and refusal weight records were maintained on daily basis during the trial period. The refusal weight left after the previous day feeding was mixed with additional feed to constitute the feed offered for the next day. The samples of the weigh back of last day of the metabolism trial were collected for nitrogen estimation, and thus the exact intake of nitrogen by the birds during trial period was estimated.

The availability of nutrients for each replicate was calculated by dividing the amount of retained nutrients (ingested nutrients – excreted nutrients) with the amount of ingested nutrients.

$$\text{Dry matter metabolizability (\%)} = \frac{\text{DM intake} - \text{DM excreted}}{\text{DM intake}} x100$$

$$\text{Nitrogen retention (\%)} = \frac{\text{Nitrogen intake} - \text{Nitrogen excreted}}{\text{Nitrogen intake}} \; x\;100$$

Determination of metabolizable energy

The gross energy of oven dried feed and excreta samples were determined by standard procedure using Digital Bomb Calorimeter. The gross heat of combustion in calories per gram of the material was computed by substituting values in the following equation.

$$\text{Gross heat of combustion (cal./g)} = \frac{T \times w - (C1 + C2 + C3)}{M}$$

Where, t, Rise in temperature; w, Water equivalent (2522 cal.); M, Weight of sample; C_1 , Correction in calories for heat of formation of acid (1.43 cal. × acid formed in ml.); C_2, Correction in calories for heat of combustion of fuse wire (2.3 cal. × length of wire used in cm.) and C_3, Correction in calories for heat of combustion of thread (27.73 cal/20 cm.).

From gross energy values of feed weigh back and excreta, the metabolizable energy (ME) was worked out by using the equation given by Hill and Anderson (1958) as below:

$$ME = E_{diet} - E_{excreta} - N \times 8.22$$

Where, ME, Metabolizable energy per kg of dry feed consumed; $E_{diet,}$ Gross energy/kg of dry feed consumed; $E_{excreta}$, Gross energy in excreta per kg of dry feed consumed and N, Nitrogen retained (g) per kg of dry feed consumed. Since it was assumed that protein tissue if oxidized for energy purposes would yield uric acid as the sole excretory product; the value, 8.22 was used as the energy value of uric acid per gram of nitrogen retained (Nitrogen correction factor).

Gross energy metabolizability

Gross energy metabolizability (%) was calculated as follows:

$$\text{Gross energy metabolizability (\%)} = \frac{\text{N corrected ME (kcal/kg)}}{\text{Gross energy of dry feed (kcal/kg)}} x100$$

Statistical analysis

The statistical analysis of data was performed using SPSS 21.0 version of Microsoft (SPSS, 2001). One way ANOVA was used for the differences between groups. When the p values were significant ($p<0.05$), a Duncan's multiple range test was performed (Duncan, D.B., 1995). All the data were expressed as mean ± standard errors.

RESULTS & DISCUSSION

Based upon the proximate composition and metabolizable energy of feed ingredients the layer's control ration was formulated as per BIS (2007) Standard. The ingredients and chemical composition of diet fed to layers in control group (T_1) is presented in table 1. The contents of crude protein, crude fiber, ether extract, nitrogen-free extract, and organic matter of basal diet (T1) were 18.04%, 4.34%, 3.61%, 66.21% and 92.20%, respectively. The calculated value of ME was 2697.17 kcal/kg feed.

Nutrients metabolizability

The present findings revealed that dry matter metabolizability (%) and nitrogen retention (%) were significantly ($P<0.05$) improved by different dietary treatments as compared to control (T_1). Further it was also observed that supplementation of salts of organic acids at all the levels of inclusion had a significant ($P<0.05$) positive effect on nitrogen corrected ME (kcal/kg) and gross energy metabolizability (%) among different dietary treatments. The value of nitrogen corrected ME (kcal/kg) and gross energy metabolizability (%) were significantly ($P<0.05$) higher in treatment T_3 (1.0% sodium-butyrate) and T_6 (1.0% calcium-propionate) as compared to all other treatments and this improvement might be due to reduction in pH of gastro-intestinal tract due to salts of organic acids and preservation of microbial balance which leads to improved metabolism and absorption of nutrients (Adam, 1999 and Hyden, 2000). These results are comparable with the earlier findings of Ghazalah *et al.* (2011) who observed that dietary organic acid supplementation significantly ($P<0.05$) increased the nitrogen retention (%), metabolizable energy (kcal/kg) and nutrients digestibility in laying hens and this improvement can be connected with greater epithelial cell proliferation in gastro-intestinal tract. Similarly, Thirumeigmanam *et al.* (2006) found a significant ($P<0.05$) improvement in ileal digestibility of nutrients and nitrogen retention (%) among different dietary treatments. In nutshell, supplementation of salts of organic acids had positive significant ($P<0.05$) effect on nutrients metabolizability.

TABLE 2: Mean values of dry matter metabolizability (%) and nitrogen retention (%) of different dietary treatments in laying hens

Treatments	Dry matter metabolizability (%)	Nitrogen retention
T_1	60.69^{a} ±0.84	60.18^{a} ±1.59
T_2	63.40^{b} ±0.95	60.78^{ab} ±2.66
T_3	66.87^{d} ±0.94	64.49^{c} ±1.46
T_4	65.64^{cd} ±1.13	63.89^{b} ±0.57
T_5	62.51^{ab} ±0.73	60.95^{ab} ±2.26
T_6	65.82^{cd} ±1.08	64.62^{c} ±1.23
T_7	64.81^{c} ±1.16	63.54^{b} ±2.01

TABLE 3: Mean values of metabolizable energy (kcal/kg feed) and gross energy metabolizability (%) of different rations in laying hens

Treatments	GE (kcal/kg dried feed)	GE (kcal/kg dried excreta)	Apparent ME (kcal/kg)	N retention/kg feed x 8.22	N corrected ME (kcal/kg)	Calculated ME	Difference	GE metabolizability (%)
T_1	4291	1445.64^{d} ±20.41	2845.36^{a} ±20.41	152.40^{d} ±3.39	2692.96^{a} ±17.46	2697.17	-4.21	62.75^{a} ±0.40
T_2	4282	1395.72^{c} ±20.29	2886.28^{bc} ±20.29	149.84^{bc} ±3.85	2736.44^{b} ±18.17	2697.17	39.27	63.90^{bc} ±0.42
T_3	4245	1304.36^{a} ±17.76	2940.64^{c} ±17.76	146.56^{a} ±1.77	2794.08^{c} ±18.19	2697.17	96.91	65.82^{d} ±0.42
T_4	4209	1328.40^{ab}±16.52	2880.60^{bc} ±16.52	148.65^{b} ±2.12	2731.95^{b} ±18.47	2697.17	34.78	64.90^{c} ±0.44
T_5	4288	1415.82^{cd} ±17.95	2872.72^{b} ±17.95	151.08^{cd} ±2.46	2721.10^{b} ±17.92	2697.17	23.93	63.45^{b} ±0.41
T_6	4256	1324.68^{ab} ±17.09	2931.32^{c} ±17.09	148.32^{b} ±1.91	2783.00^{bc} ±16.99	2697.17	85.83	65.39^{cd} ±0.39
T_7	4224	1342.18^{b} ±13.82	2881.82^{bc} ±13.82	150.64^{c} ±2.48	2731.18^{b} ±13.48	2697.17	34.01	64.91^{c} ±0.32

The hens fed the diet with high ME content presented lower feed intake, in agreement with Moraes *et al*. (1991) and Harms *et al*. (2000). This response may be explained by the fact that birds regulate their intake according to dietary energy level (Bertechini, 2006). However, when the diet contained 18% CP and 3100 kcal ME/kg, the reduction in feed intake was not pronounced, probably because, due to the higher dietary protein content, energy requirement increased. Another explanation for the lower feed intake of the hens fed 3100 kcal ME/kg was the high oil content of the feeds. Under these situations, feed passage from the gizzard to the duodenum is slower because the fat digestion in the duodenum is slow (Mateos & Sell, 1981; Mateos *et al*., 1982; Andreotti *et al*., 2004) because fats need to be emulsified, thereby reducing the appetite.

CONCLUSION

From the results of investigation, we can conclude that supplementation of sodium butyrate and calcium propionate at 1.0% level in the ration of layers, improved nitrogen corrected ME (kcal/kg) and gross energy metabolizability (%).As a conclusion of these findings, it is thought that organic acids may be beneficial when used in laying hen diets.

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