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# Effects of Sucrose-based High-lysine Diet on Blood Chemistry, Growth Performance, and Gastrointestinal Morphology of Broiler Chickens During the Growing Stage

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This study aimed to investigate the effect of replacing fat in broiler grower diet with sucrose combined with supplementation of the synthetic amino acid lysine on growth performance, gastrointestinal morphology, and blood biochemical parameters in broiler chickens. Broilers were raised for 21 days and then divided into two treatment groups ($n=24$ in each group). Two dietary treatments were used: corn-soy–based diet with oil (control) and corn-soy–based diet formulated with sucrose (3.30%) and lysine hydrochloride (3.36%). The experimental period was 21 days (from 21 to 42 days of age). At the end of week 6, all the birds in each treatment were slaughtered via neck slit, defeathered, and eviscerated for carcass and intestinal morphological characterization. Blood samples were collected to measure blood lipoprotein, triglyceride, and cholesterol levels. The results showed that supplementation of sucrose and lysine hydrochloride to broiler ration significantly ($P<0.05$) decreased feed intake by half and reduced average daily gain during the study period compared to those observed in broilers fed control diet. Further, this supplementation significantly altered gastrointestinal morphology and blood lipoprotein (HDL and LDL) and total cholesterol levels. In conclusion, corn-soy–based diet fortified with sucrose (3.30%) and lysine hydrochloride (3.36%) within current nutrient specifications has a negative effect on broiler growth performance.

**Key words**: blood chemistry, broiler, growth performance, lysine, sucrose

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## Introduction

The availability and use of dietary synthetic amino acids has allowed poultry nutritionists to reduce the dietary crude protein level, thus reducing excess nitrogen (Yadalam, 2005; Namroud *et al*., 2008), has made feed formulation easier, and has reduced feed formulation cost. Free amino acids do not require further digestion as they are rapidly and completely absorbed in the upper part of the small intestine, and to a much greater extent than amino acids derived from intact protein (Selle *et al*., 2015).

In corn- and soybean meal-based diets, lysine is considered the first and the third most limiting amino acid, respectively (Baker, 2009); thus, inclusion of synthetic lysine is necessary to enhance overall growth performance in poultry. Lysine has been supplemented at a high level (3.2 %) in corn-soybean–based, low-crude protein diet (19%) without influencing feed efficiency in male broilers during 1 to 3 weeks post hatching (Han and Baker, 1993).

Glucose has an amino acid-sparing effect, which results in increased nitrogen retention in the animal body (Fuller *et al*., 1977). In addition, glucose may reduce amino acid oxidation, which further enhances retention (Weurding *et al*., 2003). Glucose can be derived from various sources, such as sucrose. Sucrose is a disaccharide carbohydrate that can be easily utilized by poultry (Wang, 2014) and has been reported to possess a higher AMEn than glucose (3330 kcal/kg vs. 3750 kcal/kg) (Leeson and Summers, 2001). Sucrose has to be broken down into glucose and fructose by sucrase before it can be absorbed (Wang, 2014). Sugar (sucrose) has been accepted as a better energy source than starch and has been reported as a dietary energy source that could, at least partially, replace fat in poultry diets (Hussein *et al*., 2016). The different rates of absorption of amino acids and glucose by animals decrease metabolizable energy delivery to the animal body and possibly favor amino acid oxidation (van den Borne *et al*., 2007). Weurding *et al*. (2003) reported that slowly digestible starch has a sparing effect on amino acids derived from intact protein. In contrast to intact protein, synthetic amino acid is completely absorbed, at a high rate (Wu, 2009). Thus, highly absorbable synthetic amino

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acid in complete diet must be combined with an energy source characterized by complete digestion and a high absorption rate, such as sucrose.

The aim of this study was to examine the effect of replacing fat in broiler grower diet with sucrose combined with synthetic lysine supplementation on growth performance, gastrointestinal morphology, and blood biochemical parameters in broilers during the growing stage.

## Materials and Methods

### *Birds and Diet Formulation*

The animal ethics committee at the Department of Animal Production Department at Mu'tah University approved the experimental design and the procedures involved (Ref: 123/14/120).

Forty-eight broilers were raised for 21 days (from 21 to 42 days old) according to general commercial husbandry practices. Two groups of 48 broilers were randomly assigned two dietary treatments (each treatment contained 6 replicates and 4 broilers per replicate). During the experimental period, all birds were raised in floor cages and were offered one of two dietary treatments and water *ad libitum* trough a feeder and a drinker, respectively. The two dietary treatments were formulated to be isocaloric and isonitrogenous. The first diet was oil based. The second diet was sucrose-lysine based. Ingredients and chemical composition of experimental diets are shown in Table 1.

### *Sieve Analysis*

Diets were offered in a mashed form and therefore, sieving analysis was performed to evaluate the effect of ingredient composition in each diet on milled diet proprieties. Sieving analysis was carried out according to the procedure described by American Society of Agricultural and Biological Engineers (2003). Both experimental feeds were segregated by size through vertical multiple sieving under gravity with mechanical agitation, using a sieve shaker (Model No. SV001; Impact Test Equipment Ltd., Ayrshire, UK). Nine screen sieve (Impact Test Equipment Ltd.) sizes were selected to obtain a broad spectrum of particle sizes ranging from 6 mm to 0.045 mm (pan), as shown in Table 2. Experimental feeds were sieved and the geometric mean diameter ($d_{gw}$), geometric standard deviation ($S_{gw}$), surface area of milled feed, and number of particles per unit mass was determined using a 100-g sample by sieve shaking for 15 min, according to the American Society of Agricultural and Biological Engineers (2003).

### *Parameter Measurements*

Parameters were measured and data were collected as described by Al-Rabadi *et al*. (2013). At the end of the experimental period (day 42), all broilers in the experimental unit (4 broilers per replicate) were weighed for carcass and digestive system evaluation, and blood samples were collected as described by Al-Rabadi *et al*. (2015). The birds were slaughtered by cutting the jugular veins, scalded in hot water for about 1 min, and the feathers were removed by using a defeathering machine. The birds were eviscerated and weighed to obtain dressed carcass weight. The heart, gizzard, liver, spleen, abdominal fat, small intestine, and large intestine were removed and weighed using a sensitive electronic scale. The dressed carcass and digestive system organ weights were expressed as percentages of the live weights. In addition, the lengths of digestive system parts were measured using tailor's tape (accurately expressed in mm or cm).

On the day of slaughtering (day 42), blood samples were collected from the jugular vein into plain Vacutainer tubes. Samples were centrifuged at 4,000×*g* for 10 min to separate the serum, which was stored in Eppendorf tubes at −20℃ until analysis. Serum lipoproteins (total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL)), and triglyceride (TG) were analyzed using kits (Linear Chemicals S.L.,

Table 1. **Ingredients and nutrient analysis of the experimental and control diets**

| Ingredients (%) | Control diet | Sucrose + lysine diet |
|---|---|---|
| Corn | 50.04 | 63.62 |
| Soya bean meal | 27.93 | 16.55 |
| [a]Broncon concentrate | 10.50 | 11.49 |
| NaCl | 0.19 | 0.19 |
| Limestone | 1.75 | 0.89 |
| [b]Vit and mineral premix | 0.10 | 0.10 |
| Lysine hydrochloride (78%) | 0.10 | 3.36 |
| DL-Methionine | 0.23 | 0.40 |
| Degamed soybean oil | 5.27 | 0.00 |
| Monocalcium phosphate | 0.41 | 0.01 |
| Sucrose | 0.00 | 3.30 |
| [c]Antifungal | 0.10 | 0.10 |
| Wheat bran | 3.39 | 0.00 |
| [d]Chemical composition (%) | | |
| ME (Kcal/kg) | 3050 | 3050 |
| Crude protein | 21.00 | 21.00 |
| Crude fiber | 3.93 | 3.10 |
| Ca | 1.30 | 0.90 |
| Non phytate phosphorus | 0.46 | 0.35 |
| Methionine | 0.67 | 0.80 |
| Lysine | 1.25 | 4.25 |
| Cystein | 0.22 | 0.20 |
| Na | 0.22 | 0.23 |
| Cl | 0.15 | 0.15 |

[a] Brocon Concentrate® (Wafa, B V, Alblasserdam, Holland) provide (%, as on fed basis): metabolizable energy=2,200 kcal/kg; crude protein=35%; crude fiber=4.8; non-phytate phosphorus=2.2%; Methionine=1.6%; lysine=2.4%; cysteine=0.3%).
[b] Vitamin premix provided per kilogram of premix: Vitamin A, 700,000 IU; vitamin D3, 150,000 IU; vitamin E, 75 mg; vitamin B1, 100 mg; vitamin K, 175 mg; vitamin B5, 600 mg; manganese oxide, 4,000 mg, ferrous sulphate, 9,000 mg, zinc oxide, 6,000 mg, magnesium oxide, 2,500 mg, potassium iodide, 70 mg, sodium selenite, 125 mg, copper sulphate, 100 mg, cobalt sulphate, 50 mg, dicalcium phosphate, 7,000 m, sodium chloride, 10,000 mg
[c] Mold inhibitor for animal feed (Kemin Industries, USA)
[d] Calculated on the basis of analyzed values of feed ingredients (feed composition tables) from poultry NRC (1994).

Montgat, Barcelona, Spain) according to the manufacturer's recommendations. Samples were incubated for 5 min at 37 ℃, and the absorbance at 500 nm (for TG and TC) and at 600 nm (for LDL, HDL, VLDL) was read with a spectrophotometer.

***Statistical Analysis***

Statistical analysis was performed using Statistical Analysis Software (SAS, v.9.1, Institute, Cary, NC, USA). For all analyses, Student's *t*-test at the 5% significance level was used to compare the measured means between the two treatments. All data are presented as the mean (± standard deviation).

## Results and Discussion

The effects of the two diets with different ingredient compositions on $d_{gw}$, $S_{gw}$, surface area per unit mass, and number of particles per unit mass are shown in Table 2. The sucrose-lysine–based diet, when compared to the oil-based diet, had a lower $d_{gw}$ (0.96 mm *vs*. 1.13 mm, respectively), approximately similar $S_{gw}$ (2.21 *vs*. 2.09), higher surface area per unit mass (752.0 *vs*. 356.69 $cm^2$/g), and higher number of particles per unit mass (25,468.12 *vs*. 8,894.77 particles/g). Certain mash diet physical properties ($d_{gw}$ and $S_{gw}$) in complete diet have been reported to affect growth performance, eating behavior, and gastrointestinal morphology, although the effect on gastrointestinal morphology is still not clear (Amerah *et al*., 2007). However, the effect of dietary treatment on growth performance (daily gain and final body weight) in this study can be partially attributed to the reduction in feed intake. The effects of diet on broiler cumulative feed intake and average daily gain over the growing period (weeks 4–6) are shown in Table 3. Cumulative feed intake was significantly lower during the experimental period (weeks 4, 5, and 6) in the group fed the sucrose-lysine–based diet than in the animals on the oil-based diet. Overall cumulative feed intake in the sucrose-lysine–based diet group was approximately half of that in the oil-based diet-fed group (2291.9 *vs*. 4298.3 g, respectively). The lower feed intake in broilers on the sucrose-lysine–based diet could be attributed to multiple factors. First, high-level supplementation of a synthetic amino acid in the form of lysine hydrochloride in corn-soybean meal diet has been reported to reduce feed intake as a result of excess chloride provided by the lysine hydrochloride when added at more than 1.0% (Han and Baker, 1993). Urdaneta-Rincon and Leeson (2004) reported a reduction in feed intake when chicks were fed 170 g of crude protein/kg diet with 1.22% lysine, which may be related to an excess of lysine that caused a toxic effect in the chicks. Second, and in contrast to intact protein, the high absorption rate of synthetic amino acids increases ammonia levels, which may reduce broiler appetite (Namroud *et al*., 2008).

Furthermore, amino acid metabolite levels have been reported to serve as signals to regulate feed intake through an appetite-controlling mechanism (Namroud *et al*., 2008). A recent study showed that the lysine level can modify the secretion of satiety hormones (leptin and cholecystokinin), which further adjust feed intake (Yin *et al*., 2017). The absence of oil might have enhanced the dust hazard of compound feed ingredients and reduced the general appearance and palatability of the feed and thus, reduced the intake of the sucrose-lysine–based diet; this feed contains a higher number of particles per gram (Table 2).

It can be clearly seen from Table 3 that there was gradual reduction in cumulative feed intake in broilers fed sucrose-lysine–based diet in week 4 (843.3 g), week 5 (776.6 g), and week 6 (675.0 g). Average daily gain was much lower in animals fed the sucrose–lysine diet than in those on the oil-based during the experimental period (weeks 4, 5, and 6) (Table 3). Overall, average daily gain in the sucrose–lysine

Table 2. **Particles size distribution, geometric mean ($d_{gw}$), and geometric standard deviation ($S_{gw}$) of the experimental and control diets (values are presented as the means±SD)**

| Ingredients (%) | Control diet | Sucrose + lysine diet |
|---|---|---|
| Sieve size (mm) | Fraction yield | Fraction yield |
| 6 | 0.00±0.00 | 0.00±0.00 |
| 4 | 0.79±0.41 | 1.26±0.78 |
| 28 | 6.99±0.52 | 7.31±0.08 |
| 2 | 16.69±0.31 | 12.44±0.45 |
| 1 | 37.63±0.59 | 30.07±1.24 |
| 0.5 | 21.89±0.42 | 27.67±0.57 |
| 0.25 | 14.84±0.16 | 16.40±0.48 |
| 0.125 | 1.149±0.077 | 4.66±0.68 |
| 0.045 | 0.00±0.00 | 0.15±0.070 |
| Particle size parameters | | |
| ($d_{gw}$) | 1.13±0.02 | 0.96±0.01 |
| ($S_{gw}$) | 2.09±0.07 | 2.21±0.05 |
| Surface area ($cm^2$/gram) | 356.69±11.83 | 752.00±130.68 |
| Number of particles (particle/gram) | 8894.77±32.19 | 25468.12±4671.76 |

Table 3. **Effects of diet on cumulative feed intake, final body weight, and average daily gain during the experiment period (4–6 weeks of age)**

| Ingredients (%) | Control diet | Sucrose + lysine diet | *P* |
|---|---|---|---|
| Cumulative Feed intake (g) | | | |
| 1st week | 1136.3±91.1 | 843.3±64.6 | <0.0001 |
| 2nd week | 1373.3±59.6 | 776.6±66.2 | <0.0001 |
| 3rd week | 1788.8±123.8 | 675±72.0 | <0.0001 |
| Overall | 4298.3±176.7 | 2291.9±135.6 | <0.0001 |
| Average daily gain (g) | | | |
| 1st week | 79.4±8.9 | 46.0±3.2 | <0.0001 |
| 2nd week | 73.9±6.5 | 9.4±12.0 | <0.0001 |
| 3rd week | 92.6±9.8 | 9.3±19.5 | <0.0001 |
| Overall | 82.0±4.9 | 21.6±10.1 | <0.0001 |
| Initial body weight (g) | 926.5±14.7 | 921.4±18.4 | 0.6090 |
| Final body weight (g) | 2647.5±112.4 | 1374.2±205.1 | <0.0001 |

Table 4. **Effects of diet on carcass and organ weights, and tissues relative weight (%) in broilers of 42 days old**

| Parameter | Control diet | Sucrose + lysine diet | *P* |
|---|---|---|---|
| Carcass (%) | 76.72±1.67 | 68.80±2.76 | 0.0001 |
| Breast relative weight (%) | 27.97±1.40 | 20.26±3.43 | 0.0005 |
| Thigh relative weight (%) | 20.10±1.05 | 20.22±2.07 | 0.9033 |
| Gizzard relative weight (%) | 1.80±0.04 | 2.27±0.31 | 0.0152 |
| Proventriculus relative weight (%) | 0.42±0.03 | 0.44±0.05 | 0.4632 |
| Spleen relative weight (%) | 0.108±0.01 | 0.08±0.02 | 0.0845 |
| Small intestine relative weight (%) | 3.095±0.22 | 2.89±0.68 | 0.5321 |
| Large intestine relative weight (%) | 1.67±0.17 | 1.61±0.31 | 0.6973 |
| Abdominal fat relative weight (%) | 0.31±0.11 | 0.25±0.11 | 0.3342 |
| Liver relative weight (%) | 2.27±0.16 | 2.66±0.34 | 0.0333 |
| Heart relative weight (%) | 0.49±0.01 | 0.77±0.12 | 0.0024 |

diet group was approximately a quarter of that in the broilers on oil-based diet (82.0 *vs*. 21.6 g/day, respectively). The lower average daily gain in broilers fed the sucrose–lysine diet could be attributed to the lower overall feed intake. Consequently, broilers fed the experimental diet had a lower final body weight (approximately half) than broilers fed the normal diet (1,374.2 *vs*. 2,647.5 g, respectively).

All limiting amino acids (except for methionine) and their ratios to lysine were not considered when formulating the experimental diet. The decline in growth performance under the sucrose-lysine–based diet may be attributed to that the fact that glycine and/or serine became the key limiting amino acids in the reduced-protein (crude protein was reduced from 23% to 16%) corn–soybean meal diet (Baker, 2009). Amino acid-fortified low-protein corn–soybean meal diets for broiler chicks have been reported to be also deficient in threonine, arginine, and valine (Han *et al*., 1992). Insufficiency of other amino acids (other than lysine and methionine) may contribute to a reduction in broiler growth performance. Furthermore, Yadalam (2005) reported that the most common antagonism seen in poultry is that excess lysine in the diet will impair the utilization of arginine (the ratio should not be more than 1.2:1, otherwise, growth retardation may occur). However, accurate measurements of all limiting amino acids in both diets are required to confirm the influences of specific amino acids on broiler performance in this study.

It has been reported that the synchronous absorption of glucose and amino acids can increase metabolizable energy supply and may improve protein retention by decreasing amino acid oxidation (van den Borne *et al*., 2007). However, lower feed intake of the sucrose-lysine–based diet may mask the influence of presumed nutrient synchrony of the diet as a result of lower nutrient flux into the digestive system. Shortage of nitrogen pool to synthesize nonessential amino acids and insufficiency of body capacity to meet all nonessential amino acids requirements negatively influence growth performance (Namroud *et al*., 2008).

Gastrointestinal tract morphology contributes to nutrient absorption and consequently, to body weight gain (Khoa,

Table 5. **Effects of diet on the lengths of digestive system parts in broilers of 42 days old**

| Parameter | Control diet | Sucrose + lysine diet | *P* |
|---|---|---|---|
| Pancreas | 18.59±1.76 | 14.21±2.13 | 0.0031 |
| Duodenum | 40.02±3.81 | 29.41±0.90 | 0.0008 |
| Jejunum | 96.38±9.17 | 77.95±7.48 | 0.0034 |
| Ileum | 72.80±5.32 | 53.80±4.38 | <0.0001 |
| Right cecum | 24.10±2.37 | 19.51±1.62 | 0.0029 |
| Left cecum | 23.31±2.23 | 18.64±1.48 | 0.0017 |

Table 6. **Effects of diet on blood chemistry parameters in broilers of 42 days old**

| Parameter (mg/d*l*) | Control diet | Sucrose + lysine diet | *P* |
|---|---|---|---|
| TC | 163.15±16.79 | 125.46±16.44 | 0.0028 |
| HDL | 36.36±5.23 | 48.28±4.87 | 0.0022 |
| VLDL | 15.55±2.81 | 14.45±2.81 | 0.5146 |
| LDL | 112.52±15.37 | 62.71±15.62 | 0.0002 |
| TG | 77.77±14.07 | 72.27±14.08 | 0.5146 |

Abbreviations: TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; TG, triglyceride

2007). Table 4 shows the effects of the tested diets on carcass and relative organ weights. Carcass weight and breast relative weight was lower in chickens on sucrose-lysine–based diet than in animals on oil-based diet. However, gizzard, liver, and heart relative weights were higher in the sucrose–lysine diet group. No differences were found in relative weights of other organs (thigh, proventriculus, spleen, small intestine, large intestine, and abdominal fat). Along the digestive system, amino acid catabolism occurs in intestinal mucosal cells and in enterocytes to provide energy to the gut (Stoll *et al*., 1998; Wu, 1998), and this may influence conformational characteristics of the digestive system. For all parts of the digestive system evaluated (*i.e*., pancreas, duodenum, jejunum, ileum, and cecum), the organ lengths in broilers fed sucrose–lysine diet were lower than those in broilers fed the oil-based diet (Table 5). This may be explained by the lower feed intake in the broilers on the experimental diet.

The effects of sucrose-lysine supplementation on blood chemistry (lipoprotein concentrations) are shown in Table 6. Broilers fed the experimental diet had lower TC and LDL, but higher HDL levels, than to broilers fed the normal diet. A recent study by Hussein *et al*. (2016) revealed that adding 5% sugar syrup to poultry rations significantly reduced blood TC and LDL levels compared to those observed after feeding the control diet containing corn oil. Furthermore, the higher TC level in animals on the fat-based diet could be attributed to higher feed intake and to higher fatty acid intake (Hussein *et al*., 2016) derived from soy-bean oil. VLDL has been reported to be the main carrier of TG (Aliakbarpour *et al*., 2013). In this study, there were no significant differences between the two dietary treatments in terms of these two blood components. The lower feed intake in broilers fed the sucrose–lysine supplementation diet may have resulted in lower energy delivery and this may have enhanced fat lipolysis in adipose tissue, resulting in higher HDL levels. It should be mentioned that serum lipoprotein levels are genetics- and age-dependent (Piotrowska *et al*., 2011) and therefore, may widely vary, as noted in the literature (Silva *et al*., 2007; Piotrowska *et al*., 2011; Rahimi *et al*., 2015). Finally, the sucrose-lysine–based diet may negatively affect poultry meat quality due to changes in morphology and the mechanism of nutrient absorption in the digestive system (Al-Hijazeen and Al-Rabadi, 2017).

## Conclusion

Under the nutrient specifications in the current study, replacing fat in broiler grower diet with sucrose combined with synthetic lysine in low crude protein diet markedly reduced feed intake, and thus retarded growth, and altered blood lipoprotein levels and gastrointestinal morphology in broiler chickens. Further research is needed to study the effects of feeding sugar with synthetic amino acids, taking into consideration the concentrations and ratios of essential amino acids in the diet.

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## Conflicts of Interest

The authors declare no conflict of interest.

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