

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

Ghaid J. Al-Rabadi, Mustafa S. Al-Rawashdeh, Marwan A. Al-Hijazeen and Hasan Y. Al-Omari

Department of Animal Production, Faculty of Agriculture, Mutah University, Karak 61710, Jordan

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

J. Poult. Sci., 55: 263–268, 2018

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

Received: December 14, 2017, Accepted: March 14, 2018

Released Online Advance Publication: April 25, 2018

Correspondence: Marwan Abdullah Al-Hijazeen, Department of Animal Production, Faculty of Agriculture, Mutah University, Karak 61710, Jordan.
(E-mail: alhijazeenmarwan@gmail.com)

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,

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

^aBrocon 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%).

^bVitamin 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

^cMold inhibitor for animal feed (Kemin Industries, USA)

^dCalculated on the basis of analyzed values of feed ingredients (feed composition tables) from poultry NRC (1994).

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°C 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.,

Montgat, Barcelona, Spain) according to the manufacturer's recommendations. Samples were incubated for 5 min at 37 °C, 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 (\pm 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 cm²/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 cumu-

lative 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 \pm SD)

Ingredients (%)	Control diet	Sucrose + lysine diet
Sieve size (mm)	Fraction yield	Fraction yield
6	0.00 \pm 0.00	0.00 \pm 0.00
4	0.79 \pm 0.41	1.26 \pm 0.78
28	6.99 \pm 0.52	7.31 \pm 0.08
2	16.69 \pm 0.31	12.44 \pm 0.45
1	37.63 \pm 0.59	30.07 \pm 1.24
0.5	21.89 \pm 0.42	27.67 \pm 0.57
0.25	14.84 \pm 0.16	16.40 \pm 0.48
0.125	1.149 \pm 0.077	4.66 \pm 0.68
0.045	0.00 \pm 0.00	0.15 \pm 0.070
Particle size parameters		
(d_{gw})	1.13 \pm 0.02	0.96 \pm 0.01
(S_{gw})	2.09 \pm 0.07	2.21 \pm 0.05
Surface area (cm ² /gram)	356.69 \pm 11.83	752.00 \pm 130.68
Number of particles (particle/gram)	8894.77 \pm 32.19	25468.12 \pm 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)			
1 st week	1136.3±91.1	843.3±64.6	<0.0001
2 nd week	1373.3±59.6	776.6±66.2	<0.0001
3 rd 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)			
1 st week	79.4±8.9	46.0±3.2	<0.0001
2 nd week	73.9±6.5	9.4±12.0	<0.0001
3 rd 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 non-essential 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/dl)	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.

Acknowledgments

The authors thank the Deanship of Academic Research at Mu'tah University for funding this project.

Conflicts of Interest

The authors declare no conflict of interest.

References

- Al-Hijazeen MA and Al-Rabadi GJ. Dietary energy source affecting fat deposition mechanism, muscle fiber metabolic and overall meat quality. *Regulatory Mechanisms in Biosystems*, 8: 433–437. 2017.
- Amerah AM Ravindran V Lente RG and Thomas DG. Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal*, 63: 439–455. 2007.
- American Society of Agricultural and Biological Engineers. Method of Determining and Expressing Fineness of Feed Materials by Sieving. In A. S. Engineers, American Society of Agricultural and Biological Engineers Standard (pp. 601–605). St. Joseph, MI. USA. 2003.
- Aliakbarpour HR Chamani M Rahimi G Sadeghi AA and Qujeq D. Intermittent feeding programme and addition of *Bacillus subtilis* based probiotics to the diet of growing broiler chickens: Influence on growth, hepatic enzymes and serum lipid metabolites profile. *Archiv Tierzucht*, 56: 410–422. 2013.
- Al-Rabadi GJ Al-Rawashdeh MS Al-Omari HY Alkhamaiseh SK Aludatt MH and Ereifej KI. Effects of corn particle size on growth performance and on the gastrointestinal morphology of broiler chickens during growing stage. *Journal of Agriculture Science*, 11: 451–460. 2015.
- Baker DH. Advances in protein–amino acid nutrition of poultry. *Amino Acids*. Amino Acids, 37: 29–41. 2009.
- Batal AB and Parsons CM. Utilization of various carbohydrate sources as affected by age in the chick. *Poultry Science*, 83: 1140–1147. 2004.
- Fuller M F Weekes T E C Cadenhead A and Bruce J B. The protein-sparing effect of carbohydrate 2. The role of insulin. *Journal of Nutrition*, 38: 489–496. 1977.
- Han Y and Baker DH. Effects of excess methionine or lysine for broilers fed a corn-soybean meal diet. *Poultry Science*, 72: 1070–1074. 1993.
- Han Y Suzuki H Parsons CM and Baker DH. Amino acid fortification of a low protein corn–soybean meal diet for maximal weight gain and feed efficiency of the chick. *Poultry Science*, 71: 1168–1178. 1992.
- Hussein AS Al Ghurair J John PGK Habib HM Suleiman M. Graded levels of sugar syrup in broiler rations and its effect on growth performance and blood biochemical parameters. *Animal Nutrition*, 2: 180–185. 2016.
- Khoa MA. Wet and coarse diets in broiler nutrition: development of the GI tract and performance PhD Thesis, Wageningen Institute of Animal Sciences, Wageningen University and Research Centre, Wageningen, the Netherlands. 2007.
- Leeson S and Summers JD. Nutrition of the chicken, 4th edition ML Scott and Associates, Ithaca, NY. Pp 41. 2001.
- Namroudi NF Shivaazad M and Zaghari M. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poultry Science*, 87: 2250–2258. 2008.
- Nonis MK and Gous RM. Utilisation of synthetic amino acids by broiler breeder hens. *South African Journal of Animal Science*, 36: 126–134. 2005.
- Peng Y and Harper AE. Amino acid balance and food intake: Effect of different dietary amino acid patterns on the plasma amino acid pattern of rats. *Journal of Nutrition*, 100: 429–437. 1970.
- Piotrowska A Burlikowska K and Szymczko R. Changes in Blood Chemistry in Broiler Chickens during the Fattening Period, *Folia Biologica (Kraków)*, 159: 183–187. 2001.
- Rahimi S Seidavi A Sahraei M Peña Blanco F Schiavone A Androni L and Marín M. Effects of feed restriction and diet nutrient density during re-alimentation on growth performance, carcass traits, organ weight, blood parameters and the immune response of broilers. *Italian Journal of Animal Science*, 14: 583–590. 2015.
- Selle P Truong H Liu S. On free amino acids: Their role in starch and protein digestive dynamics 26th Annual Poultry Science Symposium, Sydney: Poultry Research Foundation, University of Sydney. 2015.
- Silva PRL Freitas Neto OC Laurentiz OM Junqueira AC and Fagliari JJ. Blood serum components and serum protein test of Hydro-PG broilers of different ages. *Brazilian Journal of Poultry Science*, 9: 229–232. 2007.
- Stoll B Henry J Reeds PJ Yu H Jahoor F and Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *Journal of Nutrition*, 128: 606–614. 1998.
- Urdaneta-Rincon M and Leeson S. Muscle (Pectoralis Major) protein turnover in young broiler chickens fed graded levels of lysine and crude protein. *Poultry Science*, 83: 1897–1903. 2004.
- van den Borne JJGC Schrama JW Heetkamp MJW Verstegen MWA Gerrits WJJ. Synchronising the availability of amino acids and glucose increases protein retention in pigs. *Animal*, 1: 666–674. 2007.
- Wang A. The effects of different feeding program and inclusion of glycerol, glucose or sucrose in broiler starter diets on growth performance and intestinal development Master thesis Dalhousie University, Halifax, Nova Scotia, Canada. 2014.
- Weurding RE Enting H and Verstegen MWA. The relation between starchdigestion rate and amino acid level for broiler chickens. *Poultry Science*, 82: 279–284. 2003.
- Wu G. Intestinal mucosal amino acid catabolism. *Journal of Nutrition*, 128: 1249–1252. 1998.
- WU G. Amino acids: metabolism, functions, and nutrition. *Amino Acids*, 37: 1–17. 2009.
- Yadalam S. Modeling broiler energy and protein metabolism. PhD Thesis Faculty of the Graduate College, Oklahoma State University, Oklahoma, USA. 2005.
- Yang X Zhuang J Rao K Li X and Zhao R. Effect of early feed restriction on hepatic lipid metabolism and expression of lipogenic genes in broiler chickens Research in Veterinary Science, 89: 438–444. 2010.
- Yin J Han H Li Y Liu Z Zhao Y Fang R Huang X Zheng Ren W Wu Liu G Wu X Wang K Sun L Li C Li T Yin Y. Lysine Restriction Affects Feed Intake and Amino Acid Metabolism via Gut Microbiome in Piglets. *Cell Physiology and Biochemistry*, 44: 1749–1761. 2017.