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The effect of glibenclamide on performance, carcass characteristics, blood parameters, immunity, intestinal microbial flora, intestinal morphology and breast muscle fatty acid profile in broilers

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Abstract

Introduction: Glibenclamide, with IUPAC name chloro-N-(4-[N-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide, is a sulfonylurea derivative with a melting point of 169-174 ◦C. This substance is in the form of a white crystalline powder, often without smell and taste, insoluble in water but soluble in methylene chloride. Glibenclamide is classified as sulfonylurea drugs which act by stimulating beta cells of the pancreas to secrete more insulin through increased intercellular cAMP. These drugs are only effective in people who have minimal ability to secrete insulin. At the same time, these drugs have been suggested to increase the binding of insulin to receptors and increase the number of insulin receptors (Rambiritch et al. 2007). Recent studies have reported that glibenclamide also plays a role in the hypoglycemic effects in the regulation of inflammation. No data are available regarding the effect of glibenclamide in broilers and whether glibenclamide can have an effect on performance, carcass characteristics, blood parameters, immunity, intestinal microbial flora, intestinal morphology, and fatty acid profile and not clear whether it is benefit in chicken or not and it needs further investigation.

Materials and methods: This research was conducted in 2022 at a broiler farm located in Rasht. The trial utilized 120 one-day-old broiler chickens of the commercial strain Ross 308 with an average weight of 43±1g. The experiment was conducted as a completely randomized design with 3 treatments and 4 replications of 10 chickens per pen for 42 days. Experimental treatments include three dietary concentrations of glibenclamide: 0 (Control), 75, or 100 mg/kg glibenclamide). The ration was formulated to meet the minimum nutritional requirements of the Ross 308 strain. Chickens were reared in 1×1 m cages on a bed of cellulose rolls for 42 days. The weight gain of all chickens in each pen was calculated by a digital scale with an accuracy of ±10 g during periods of 1 to 10, 11 to 21, and 22 to 42 d. At the end of each period, the amount of remaining feed (beginning 1 to 10, growth 11 to 21 and ending 22 to 42) at the beginning of each period, the amount of feed consumed was calculated by weighing in each feeder and deducting from the amount of feed given. Also, feed conversion was calculated by dividing the amount of feed consumption by the weight gain for days 1 to 10, 11 to 21, and 22 to 42 and the entire period. Economic efficiency, carcass characteristics, digestive organs, blood parameters, immune responses, intestinal microbial flora, intestinal morphology, and breast fatty acid profile were measured. All data collected during the

experiment and laboratory traits were analyzed by analyses of variance using statistical software. Means were compared with Duncan's multiple range test at 5% statistical levels.

Results and discussion: The results indicate that during all of the growing periods, the use of glibenclamide did not affect the performance of broiler chickens ($P \geq 0.05$). Also, weight at 42 d of age, feed cost per kg live weight (Rial/kg), and European index were not different among dietary concentrations of glibenclamide ($P \geq 0.05$). Use of two different levels of glibenclamide did not have a difference on live weight, featherless weight, thigh percentage, breast percentage and abdominal fat ($P \geq 0.05$), but 75 mg/kg was reported to reduce fat in the ventricular area. Glibenclamide did not affect blood glucose concentrations ($P > 0.05$). Feeding 0 or 75 mg/kg of glibenclamide as a sulfonylurea derivative reduced cholesterol, triglycerides, HDL, VLDL and LDL concentrations ($P < 0.05$). The LDL to HDL ratio was highest for 75 compared with 0 and 100 mg/kg glibenclamide ($P < 0.05$). In contrast, concentrations of total protein, albumin and globulin were greatest for 0 mg/kg, intermediate for 75 mg/kg and highest for 100 mg/kg ($P < 0.05$). No differences were observed among treatments on humoral immune system function in response to SRBC antigen injection, except for 35 d which was highest for 100 mg/kg glibenclamide, and antibody titer against Newcastle virus and influenza (Table 7). In response to SRBC antigen injection, Newcastle and influenza titers increased at the end of the course. Feeding glibenclamide did not increase the relative weight of the bursa of Fabricius or the weight of the spleen ($P \geq 0.05$). Feeding 0 or 75 mg/kg glibenclamide supported lower numbers of Escherichia coli bacteria compared to other treatments ($P < 0.05$); however, no differences were observed in the number of Lactobacillus acidophilus. The highest villi length was observed with the consumption of 75 mg/kg glibenclamide. The depth of the crypt increased in the same dose of glibenclamide compared to the control group, but the ratio of villi length to the depth of the crypt decreased. The results indicate that lower percentages of myristic and palmitic acid were observed when 75 mg/kg of glibenclamide was fed compared with 100 mg/kg; however, palmitoleic, steric, oleic, linoleic, and linolenic acid percentages were lowest and the ratio of saturated to unsaturated fatty acids was highest when 100 kg/kg of glibenclamide was fed. In general, the results of this trial indicate that glibenclamide does not improved feed consumption, weight gain, conversion rate, total cost per kilogram of live chicken and production index in the rearing period. However, feeding low concentrations could reduce abdominal fat. Feeding low concentrations of glibenclamide led to a positive effect on blood factors, including triglycerides, cholesterol, VLDL, LDL, total protein. Populations of Escherichia coli were reduced in the intestine which appeared to improve villi length and crypt depth as well as the ratio of villi length to crypt depth compared to the control group. In comparison of saturated and unsaturated fatty acids, its low dose was able to reduce the ratio of saturated to unsaturated fatty acids.

Conclusion: Therefore, according to the positive effects of glibenclamide on carcass characteristics, blood parameters, immunity, intestinal flora, intestinal morphology and fatty acid profile, this food additive can be used as an alternative to commercial antibiotics and a cheap growth promoter.

Key words: Bifidobacterium, Cholesterol, drumstick, growth, immunity, villi

Introduction

Glibenclamide, with IUPAC name chloro-N-(4-[N-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide, is a sulfonylurea derivative. This substance is in the form of a white crystalline powder, often without smell and taste, insoluble in water but soluble in methylene chloride. Glibenclamide is classified as sulfonylurea drugs which act by stimulating beta cells of the pancreas to secrete more insulin through by increasing intercellular cyclic adenosine monophosphate (cAMP). These drugs are only effective in people who have minimal ability to secrete insulin (Henquin, 2021). At the same time, these drugs have been suggested to increase the binding of insulin to receptors and increase the number of insulin receptors (Rambiritch *et al*. 2007). Recent studies have reported that glibenclamide also has a hypoglycemic effect in regulation of inflammation. Glibenclamide also inhibits Sur1-Trpm4 channels by directly binding to the Sur1 subunit to protect against inflammation-related damage in the central nervous system (CNS) (Ghosian *et al*. 2015). Activation of Sur1-Trpm4 channels depolarizes the cell membrane, which leads to cell death (Larypoor *et al*. 2020). Glibenclamide leads to a reduction of adverse neuroinflammation and behavioral consequences in central nervous system damage (Mohseni *et al*. 2022). In addition, it plays reduces damage caused by inflammation in various systems, including respiratory (Visavadiya *et al*. 2009; Mirershadi *et al*. 2015), gastroenterology (Abbas *et al*. 2015), urology (Al-Qarawi *et al*. 2002; Al-Daraji *et al*. 2012), cardiology (Al-Razzuqi *et al*. 2012), the central nervous system (Armanini *et al*. 2003; Al-Snafi 2018), some specific conditions such as melioidosis (Armanini *et al*. 2002; Visavadiya *et al*. 2009), ischemic reperfusion injury (IR) (Awadein *et al*. 2010; Arvelo *et al*. 2015) and septic shock (Baker. 1994; Aziz *et al*. 2018). Bronchopulmonary dysplasia is a devastating lung complication in premature infants and inflammation plays an important role in its development (Ryan *et al*. 2008; Benko *et al*. 2008) which should study in poultry. A group of researchers reported that glibenclamide protects newborn mice from developing bronchopulmonary dysplasia. In fact, glibenclamide inhibits activation of caspase-1, reducing the production of interleukin-1beta and suppressing the influx of neutrophils and macrophages (Liao. 2018). As a result, the recovery and general health of the mice increased. Although the pathogenesis of acute pancreatic necrosis has not yet been clarified, inflammatory mediators such as IL-1β, IL-6, IL-8, IL-10, tumor necrosis factor alpha-α (TNF), platelet-activating factor, and monocyte chemotactic protein 1 MCP-1) are considered critical for the development of acute pancreatic necrosis (Li *et al*. 2015). Glibenclamide inhibited lipopolysaccharide-induced IL-1β release in peritoneal cells in laboratory conditions (York *et al*. 2014). The activity of glibenclamide in lowering blood glucose remains unchanged when used with cimitidine, while plasma glucose concentration was higher when glibenclamide is used with cimitidine or ranitidine (Donahue *et al*. 2002).

Examining the effect of metformin on the Advanced glycation end products (AGE) receptor and HMGB1 in the myocytes of hyperglycemic rats, they concluded that metformin protects against cardiovascular damage caused by hyperglycemia by inhibiting the expression of He became the recipient of advanced glycation products and box 1 protein group. Therefore, the results of their studies showed that metformin may reduce cardiovascular damage caused by hyperglycemia by inhibiting the expression of RAGE and HMGB1 (Zhang et al. 2014).

Erejuwa et al. (2011) compared antioxidant effect of honey, glibenclamide, metformin and their compounds in STZ-induced diabetic rats and reported that oxidative damage was not changed by treatment with metformin or glibenclamide, but the combination of the two drugs with honey had very good antioxidant effects. These effects may be due to the regulation of renal oxidant levels.

Adeshara and Tupe. (2016) reported that treatment with metformin and glipizide against glycated albumin inhibited the formation of glycation products and eliminated the structural changes. These compounds returned the antioxidant levels to the first level and had a protective effect on the cell. The results of their study finally show the protective effect of this compound against glycation albumin, can prevent cell damage caused by glycation, and return the level of antioxidant defense to the original state.

There are few reports about glibenclamide feeding in broilers (Felipa et al, 2011; Al-Sultany et al. 2013) and hens (Codreanu et al. 2009). Al-Sultany et al. (2013) found there is no significant difference between groups doses of glibenclamide and all concentrations although superiority the 2nd and the 3rd groups by weight on the 1st and control groups in the 5th week, and the superiority of the 2nd group weight on the rest of the groups, in addition to superiority over the control group in the 6th week of the experiment carcass weight and the ratio of meat net between the in meat chicken in addition to the control group, indicating a good feed conversion ratio and construction processes within the body and the production of meat when a chicken to focus 0.2 mg/ml of the glibenclamide. There is no significant difference in both the relative weights of carcass parts among all groups studied compared to a control sample. There are no significant differences between groups meat chicken used in the experiment and dosages concentrations of the three glibenclamide compared to control group. This indicates that the glibenclamide did not affect the increase weighted for meat chicken, but the effect was evident in amount feed intake and feed conversion ratio. The drug did not affect the increase weighted for meat chicken, but the effect was evident in the amount of intake feed and feed conversion ratio, demonstrates the good feed conversion ratio and construction processes within the body and the production of meat when a chicken to the concentration of 0.2 mg/ml of the glibenclamide drug. Although there are few studies on glibenclamide feeding in broilers, there are new findings about other novel additives feeding in poultry farming (Phillips et al. 2023; Seidavi et al. 2023).

Based on available literature, there are positive effects of glibenclamide biochemically and physiologically in humans and since no data are available regarding the effect of glibenclamide in broilers and we do not know whether glibenclamide can have a positive effect on broiler performance, carcass characteristics, blood parameters, immunity, intestinal microbial flora, intestinal morphology, and fatty acid profile, this research project was organized and conducted.

Materials and Methods

This research was conducted in 2022 at a broiler farm located in Rasht. The trial utilized 120 one-day-old broiler chickens of the commercial strain Ross 308 with an average weight of 43±1g. The experiment was conducted as a completely randomized design with 3 treatments and 4 replications of 10 chickens per pen for 42 days. Experimental treatments include three dietary concentrations of glibenclamide: 0 (Control), 75, or 100 mg/kg glibenclamide (Iran-Darou Co. Mashhad, Iran) into basal ration based on our preliminary trials. The experimental diets were formulated to meet the minimum nutritional requirements of the Ross 308 strain (Manual. 2012, Table 1). Chickens were reared in 1×1 m cage on a litter of cellulose rolls for 42 days. The temperature in the breeding hall in the first week was maintained at 33 ◦C and then gradually decreased to 23 ◦C on the 18th day of the experiment and maintained at that temperature until the end of the trial. The environmental conditions for all chickens were similar and included 23 hours of light exposure and 1 hour of darkness with a humidity of 65 to 70%. Free access to water and feed during the rearing period was provided for all cages. The birds were

vaccinated against infectious bronchitis (d 10 of age), Newcastle (d 4, 21 and 35 of age) and Infectious Bursal disease (d 12 of age). All vaccines were obtained from Razi Serum and Vaccination Institute (Karaj, Iran).

Growth performance and economic efficiency

The weight gain of all chickens in each pen was measured by a digital scale (Tozin Kala, Iran) with an accuracy of ±10 g during periods of 1 to 10, 11 to 21, and 22 to 42 d. At the end of each period, the amount of remaining feed (beginning 1 to 10, growth 11 to 21 and ending 22 to 42) at the beginning of each period, the amount of feed consumed was calculated by weighing in each feeder and deducting from the amount of feed given.

Table 1. Ingredients, chemical composition, and energy of the used diets (from 1 to 42 d of age).

Ingredients (%, as-fed)	Starter (1-10 d)	Grower (11-21 d)	Finisher (22-42 d)
Corn	47.03	59.60	65.99
Wheat	5.58	5.00	5.00
Soybean meal (44% Crude protein)	29.02	16.15	10.28
Corn gluten	10.00	11.48	11.50
Soy oil	3.50	3.40	3.09
Limestone	1.45	1.23	1.00
Di-calcium phosphate	1.95	1.80	1.83
Salt	0.20	0.20	0.20
Vitamin and mineral supplements[1]	0.50	0.50	0.50
DL-methionine	0.52	0.58	0.57
L-lysine hydrochloride	0.25	0.06	0.04
Calculated compounds			
Metabolizable energy (kcal/kg)	2950	3000	3050
Crude protein (%)	22	20	19
Lysine (%)	1.3	1.2	1.1
Methionine (%)	0.56	0.54	0.52
Met+Cys (%)	0.92	0.90	0.88
Calcium (%)	1.04	0.95	0.92
Available phosphorus	0.52	0.47	0.41

[1]Vitamins and mineral supplement contained (per kg of the final diet): 9000 IU vitamin A; 3000 IU vitamin D3; 18 IU vitamin E; 3 mg vitamin K3; 1.8 mg vitamin B1 (Thiamine); 6 mg vitamin B2 (Riboflavin); 3 mg vitamin B6 (Pyridoxine); 0.012 mg vitamin B12 (Cyanocobalamin); 30 mg vitamin B3 (Niacin); 1 mg vitamin B9 (Folic acid); 0.24 mg vitamin H3 (Biotin); 10 mg vitamin B5 (Pantothenic acid); 500 mg choline,100 mg Mn; 100 mg Zinc; 80 mg Iron; 10 mg Cu; 1 mg I; 0.2 mg Selenium

Also, feed conversion was calculated by dividing the amount of feed consumption by the weight gain for days 1 to 10, 11 to 21, and 22 to 42 and the entire period (Sigolo *et al*. 2019).

The following formula was used to measure the European production index:

European production index = 10 × number of rearing days × feed conversion ratio / retention percentage ×average live weight (grams)

In order to measure the cost of feed consumed per kilogram of live chicken, the following formula was used. The daily price of used glibenclamide was calculated separately for each ration and inserted into the formula.

Cost of feed consumed per kilogram of live chicken = (Weight of a chicken at 42 d in kilograms/price of feed consumed during 42 d for each chicken in Rials)

Carcass characteristics, digestive organs

After 2 h of feed withdrawal, at the end of the experiment, 2 birds from each replication with a weight close to the average were selected and slaughtered. The live body weight, defeather body weight, carcass weight, breast weight, thigh weight, wing and neck weight, as well as the weight of internal organs (heart, spleen, bursa of Fabricius, liver, abdominal fat) was determined using a digital scale (AandD GF-300 digital scale balance (310 g × 0.001 g, A and D Weighing Design and Manufacture, San Jose, CA) with an accuracy of 0.01 g) (Naddaf-Fahmideh *et al*. 2023).

Blood parameters

At the end of the experiment (42 d), 2 birds with close to the average weight were randomly selected from each from each replicate and 5 ml of blood was collected from the wing vein. Samples were combine by replicate and maintained for 12 h at room temperature to separate the serum; afterwhich, the samples were centrifuged (Eppendorf 5702, Germany) at 5000 rpm for 3 min, serum transferred to microtubes, and then transported to the laboratory (Viro-Med, Rasht, Iran) where it was s at minus 20 ◦C. Serum was thawed at room temperature for analyses of glucose, triglyceride, cholesterol, total protein, albumin, globulin, *very-low-density lipoprotein* (VLDL), *high-density lipoprotein* (HDL, LDL) using Pars Azmoun commercial kits and by an autoanalyzer (Hitachi 917, Japan) according to the method (Golrokh *et al*. 2016).

Immune responses

Broiler chickens were immunized against sheep red blood cells (SRBC) according to Lemer's method to check humoral immunity (Lerner *et al*. 1971). Blood was collected from 3 sheep and SRBC suspension was prepared by washing three times in PBS saline phosphate buffer before preparing a final suspension of 2% SRBC in PBS. On d 28 and 36, 0.1 cc of the 2% SRBC solution was injected into the wing vein of 2 birds from each replicate. Blood samples were collected on d 35 and 42 (Gore and Qureshi. 1997). The amount of antibody against SRBC was measured by hemagglutination method. Special pellets V microhemagglutination was prepared to measure the antibody titer. Then the Van derzipp (Van der Zipp et al. 1983) method was used to measure the total antibody. According to this method, 50 ul of serum was mixed with 50 ul of phosphate buffer to measure the total anti-SRBC. Saline (PBS) was mixed inside the microtiter plate and serial dilutions from 1.2 to 1.256 were prepared from serum. Titers were expressed based on Log2, the highest dilution that exhibited complete agglutination (Pourhossein *et al*. 2015). In order to measure the titer of Newcastle (NDV) and influenza on d 28 and 42 from each pen, blood was collected from 2 birds and combine and hemagglutination inhibition test was performed on the samples. The hemagglutination inhibition test was based on OIE (Office international des epizooties) standard. To perform the test, a 96-well microplate containing 4 units of antigen (Pasouk, Iran) was used. Dilution of titers was conducted based on log2. The 1% red blood cells used were prepared from Sun Protection Factor chickens. In order to investigate the effect of glibenclamide on the immune system, at the end of the experiment, after two hours of feed withdrawal, 2 birds from each replicate with a weight close to the average were slaughtered and the weight of the spleen and bursa of Fabricius was measured using a digital scale as described previously (Naddaf-Fahmideh *et al*. 2023).

Intestinal microbial flora

On d 42, two birds from each replicate were slaughtered and the abdominal cavity was opened, then the right and left cecum were separated with sterile scissors and their contents emptied into sterile microtubes and to measure the population of *Escherichia coli*, *lactobacillus acidophilus* Bifidobacterium were stored at -20°C until the microbial culture according to Dibaji *et al*. (2014) in laboratory (Viro-Med, Rasht, Iran). Serial dilutions (1 to 10 ratio) using distilled water. Diluted samples were autoclaved at 120 degrees' atmospheric pressure. For this purpose, one gram of each of sample was added to 9 mL of distilled water to form dilution series from 1-10 to 10-6. Then 300 µL of each dilution series of 10-3, 10-4, and 10-5 were taken and completely spread on the culture surface. Cultivation was conducted next to the flame and under the hood. The inoculated samples in were incubated at 37 ◦C for 24 h for the growth of *Escherichia coli* and *lactobacillus acidophilus* bacteria in the culture medium (EMB Eosin methylene blue Agar) and Bifidobacterium bacteria in the culture medium (Blood Agar BA) (Jang *et al*. 2007). In order to determine CFU (Colony Forming Units), the colonies formed were counted in the most suitable dilution (4-10). After counting, the number of colonies on each culture medium was multiplied by the dilution ratio. Considering the magnitude of the numbers obtained from bacteria counting, in order to simplify calculations, the logarithm of the numbers mentioned in base 10 was calculated for data analysis (Hosseintabar *et al*. 2014; Dibaji *et al*. 2014).

Intestinal morphology

Intestinal morphology was measure in 1 bird from each treatment slaughtered at 42 d. Tissue sections from birds slaughtered at 42 d of age were cut from the intestine (1 cm from the middle part of the intestinal jejunum) and placed inside the vials containing 10% formalin and transferred into laboratory (Viro-Med, Rasht, Iran). After three steps of changing formalin and fixing the tissue samples, 5 mm sections were cut with a sterile surgical blade and prepared by the hematoxylin and eosin (HandE) method. The usual HandE staining steps include, respectively, floating in two tanks. Gesylol (10 min each), 96% alcohol (5 min), 100% alcohol (5 min) and hematoxylin (10 min) once by inserting alcohol into the acid container and then washing three steps with distilled water, eosin (3 min), and putting in 96% and 100% alcohol in the container and finally clarification was completed in two Gesylol containers. Using a microscope, the height of the villi and the depth of the crypt were examined as outlined by Kalantari-Hesari (2022). Histomorphometric indicators were studied using the hematoxylin-eosin staining method in the jejunum which include the thickness of the entire intestinal wall from the base of the hairs to the serous layer, the length of the hairs, the thickness of the hairs, the ratio The length to the depth of the crypts was the thickness of the epithelium.

Breast fatty acid profile

The fatty acid profile of breast meat was measure in 1 bird from each treatment slaughtered at 42 d. Fat samples were collected and mixed with 100 ml of methanol solution of chloroform (2:1) about 3 to 4 times. Then was well mixed. The filtered samples were mixed with 25 ml saturated sodium chloride solution in a decanter funnel. The chloroform phase containing fat was smoothed using filter paper impregnated with anhydrous potassium sulfate and then dried by a rotating operator under vacuum until only the fat remained. Then, 10 mg of extracted fat was mixed well with 2 ml of potassium hydroxide, 2 ml of normal methanol and 7 ml of n-hexane and the resulting sample centrifuged for 10 min. The centrifuged sample was allowed to stand for 5 min to separate the upper phase. Sepsis, about one microliter of the upper phase was injected to evaluate the profile of fatty acids inside the gas chromatograph (Series B GC7890, Agilent America) and the concentration of fatty acids expressed as a percentage (Folch *et al*. 1957).

Statistical Analysis

All data collected during the experiment and laboratory traits were analyzed by analyses of variance using statistical software (SAS, 2001) based on a completely randomized design (CRD) using following formula:

$Yij=\mu+\alpha i+\varepsilon ij$

Yij= number of observations in the experiment

μ= mean

αi= effect of each treatment

εij= effect of experimental error

Means were compared with Duncan's multiple range test at 5% statistical levels.

Results and Discussion

Performance

The effect of different concentrations of glibenclamide on the performance of broiler chickens is presented in Tables 2, 3 and 4. The results indicate that during all of the growing periods, the use of glibenclamide did not affect the performance of broiler chickens ($P\geq0.05$). Also, weight at 42 d of age, feed cost per kg live weight (Rial/kg), and European index were not different among dietary concentrations of glibenclamide ($P\geq0.05$). Zhang *et al*. (2014) reported that metformin protected against cardiovascular damage caused by hyperglycemia by inhibiting the expression of RAGE and the expression of the glycation end product receptor. In the present study, which was conducted on type 2 diabetic patients, the group receiving metformin had a decreasing effect on RAGE expression, and the group of patients receiving metformin and glibenclamide had a greater decreasing effect than the previous group (the group receiving metformin alone). The present study is in line with the results of this research. In another study, a group of researchers found that the combined treatment of metformin and insulin on systemic liver changes in a diabetic model induced by a high-fat diet is far more effective. It has had a better effect than insulin treatment and both drugs with the help of each other have had a reducing effect on vascular damage and liver disorders caused by diabetes, and also metformin together with insulin improved liver and systemic injury. Along with glibenclamide, it has a better and more appropriate reducing effect than metformin only on type 2 diabetic patients compared to the control group of type 2 diabetics without receiving the drug, as a result, the simultaneous treatment of both metformin and glibenclamide has a reducing effect on RAGE expression. which shows its positive effect compared to metformin alone.

Characteristics of carcass, carcass fat and digestive organs

The effect of experimental treatments on carcass characteristics is shown in Table 5. The results showed that the use of two different levels of glibenclamide did not have a difference on live weight, featherless weight, thigh percentage, breast percentage and abdominal fat ($P\geq0.05$), but 75 mg/kg was reported to reduce fat in the ventricular area (Tanaka *et al*. 1999). Considering effect of metformin on the formation of glycation end product and peripheral effect on diabetic rats, researchers concluded that metformin exerts its anticoagulant effect directly and improves the function of peripheral nerves. As a result, metformin is effective in inhibiting the effects and complications of diabetes by reducing the amount of blood glucose through inhibition of the production of stable products in the glycation process. Glibenclamide is the second generation of hypoglycemic sulfonylureas. Sulfonylurea stimulates insulin secretion from beta cells in the pancreas and can lead to hypoglycemia (Hanrahan *et al*. 2011). Insulin has been shown to have effects on carbohydrate, lipid, and protein metabolism during embryonic and post-hatch periods. It also stimulates the transfer of glucose and amino acid in different cells. However, the effect of insulin in the transfer of glucose in muscles is limited and it is still doubtful in the transfer of glucose to adipose tissue of chicken. Insulin stores proteins mainly by increasing the entry of amino acids (especially valine, leucine, isoleucine, tyrosine and phenylalanine) into

the cell, stimulating the translation of mRNA and transcription from DNA to make proteins, inhibiting the breakdown of proteins, and reducing the rate of gluconeogenesis. An important point related to the effect of insulin-retaining protein is that the presence of insulin is necessary to advance the effects of growth hormone in increasing the body tissue growth and without its presence, growth hormone cannot stimulate growth (Guyton and Hall. 2011). York *et al*. (2014) reported that glibenclamide reduces serum concentrations of IL-6, lipase and amylase in mice with pancreatic necrosis caused by cerulein.

Table 2: Growth performance of broilers at three stages of growth fed diets containing different concentrations of glibenclamide

Glibenclamide (mg/kg)	1 to 10 d			11 to 21 d			22 to 42d		
	Feed intake (g/chick/d)	Weight gain (g/chick/d)	Feed conversion ratio	Feed intake (g/chick/d)	Weight gain (g/chick/d)	Feed conversion ratio	Feed intake (g/chick/d)	Weight gain (g/chick/d)	Feed conversion ratio
0	24.125	21.600	1.119	69.825	48.932	1.427	130.438	70.963	1.839
75	24.275	22.375	1.085	71.475	49.636	1.440	133.928	74.000	1.810
100	24.325	22.450	1.084	69.375	49.068	1.417	129.922	70.875	1.834
P-value	0.979	0.711	0.104	0.692	0.940	0.535	0.319	0.116	0.617
SEM	0.722	0.791	0.011	1.784	1.491	0.015	1.910	1.071	0.022

[a,b] Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means

Table 3: Growth performance of broilers fed diets containing different concentrations of glibenclamide

Glibenclamide (mg/kg)	1 to 42 d of age		
	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio
0	88.416	53.012	1.668
75	90.602	54.872	1.651
100	88.091	53.214	1.656
P-value	0.432	0.235	0.671
SEM	1.422	0.781	0.013

[a,b]Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means

Table 4: Economical performance of broilers at 42 d of age fed diets containing different concentrations of glibenclamide

Glibenclamide (mg/kg)	Weight 1 chick at 42th days of age (gr/chick)	European production index	Total cost ($/m²)	Total income ($/m²)	Profit ($/m²)
0	2220.000	324.777	24.958	32.225	7.268
75	2296.250	339.219	25.580	33.333	7.753
100	2228.250	328.332	24.877	32.342	7.467
P-value	0.235	0.220	0.435	0.234	0.379
SEM	32.036	5.610	0.402	0.464	0.234

[a,b]Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means

Blood parameters

The results of different concentrations of glibenclamide on blood parameters are reported in Table 6. Glibenclamide did not affect blood glucose concentrations (P>0.05). Kagami *et al*. (2008) reported that caffeine has a protective effect on pancreatic beta cells and can prevent their destruction, glibenclamide increases insulin secretion from pancreatic beta cells appears, and caffeine can reduce blood sugar concentrations only in the presence of insulin induced by glibenclamide, while caffeine was not effective (Wright *et al*. 2004). The use of metformin alone or together with sulfonylureas such as glibenclamide led to blood glucose control in type 2 diabetes patients (Gilani and Feizabad. 2019). In fact, the main mechanism of action of metformin such as glibenclamide is inhibition of hepatic gluconeogenesis and glycogenolysis and increased sensitivity of peripheral tissues to insulin (Gilani and Feizabad. 2019).

Feeding 0 or 75 mg/kg of glibenclamide as a sulfonylurea derivative reduced cholesterol, triglycerides, HDL, VLDL and LDL concentrations (P<0.05). The LDL to HDL ratio was highest for 75 compared with 0 and 100 mg/kg glibenclamide (P<0.05). In contrast, concentrations of total protein, albumin and globulin were greatest for 0 mg/kg, intermediate for 75 mg/kg and highest for 100 mg/kg (P<0.05). Silvares *et al*. (2016) stated that a combination of metformin and insulin was more effective than insulin on hepatic systemic changes in a diabetic model induced by a high fat diet. These researchers also reported that metformin together with insulin could improve liver and systemic injury caused by diabetes. The combined treatment of glibenclamide and caffeine improved serum glucose control, which can lead to significant and beneficial changes in the serum triglyceride and HDL levels of diabetic rats (Qousian Moghadam *et al*. 2014; Ghosian *et al*. 2015).

Table 5: Means weight and relative weight of invaluable body parts of broilers at 42 d of age fed diets containing different concentrations of glibenclamide

Glibenclamide (mg/kg)	Live body weight (gr)	Defeather body weight (gr)	Relative weight of breast (%)	Relative weight of drumsticks (%)	Relative weight of wings (%)	Relative weight of abdominal fat (%)	Relative weight of heart (%)	Relative weight of neck (%)
0	2411.250	1540.000	25.750	19.750	4.455 [b]	0.920	0.390	2.472
75	2397.750	1553.750	25.750	19.500	5.638 [a]	0.680	0.430	2.552
100	2356.250	1555.000	26.750	20.000	5.375 [a]	0.955	0.445	2.637
P-value	0.768	0.952	0.625	0.894	0.036	0.326	0.157	0.626
SEM	55.012	37.238	0.821	0.741	0.280	0.133	0.019	0.117

[a,b] Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means

Table 6: Means of blood constitutes of broilers at 42 d of age fed diets containing different concentrations of glibenclamide

Glibenclamide (mg/kg)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDL/HDL ratio	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
0	241.250	122.500 [b]	72.825 [b]	14.565 [b]	38.975 [b]	64.300 [b]	1.652 [a]	3.150 [a]	1.155 [a]	1.995 [a]
75	240.750	122.000 [b]	72.075 [b]	14.415 [b]	40.000 [b]	61.650 [b]	1.545 [b]	2.828 [b]	1.058 [b]	1.773 [b]
100	242.250	145.000 [a]	86.300 [a]	17.260 [a]	45.750 [a]	76.975 [a]	1.678 [a]	2.650 [c]	0.990 [c]	1.660 [c]
P-value	0.838	<0.0001	0.000	0.000	0.001	<0.0001	0.006	<0.0001	<0.0001	<0.0001
SEM	1.797	1.893	1.468	0.294	0.861	1.123	0.023	0.051	0.016	0.036

[,b] Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means; VLDL: Very low-density lipoprotein; HDL: High density lipoprotein; LDL: Low density lipoprotein

Table 7: Mean immune response of broilers at 42 d of age fed diets containing different concentrations of glibenclamide

Glibenclamide (mg/kg)	Newcastle disease (log2)		Influenza disease (log2)		Sheep red blood cell (log2)	
	Step one	Step two	Step one	Step two	Step one	Step two
0	3.000	3.250	2.500	4.500	2.500 [b]	1.000
75	3.000	4.000	3.250	5.250	4.250 [ab]	1.750
100	4.750	1.750	2.750	4.500	6.000 [a]	1.250
P-value	0.079	0.054	0.178	0.291	0.006	0.075
SEM	0.546	0.565	0.264	0.363	0.571	0.204

[a,b] Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means

Table 8: Mean relative weight of organs related with immune system of broilers at 42 d of age fed diets containing different levels of glibenclamide

Glibenclamide (mg/kg)	Relative weight of liver (%)	Relative weight of spleen (%)	Relative weight of bursa of fabricius (%)
0	2.090	0.135	0.103
75	1.779	0.112	0.155
100	2.182	0.147	0.182
P-value	0.456	0.174	0.740
SEM	0.228	0.012	0.073

[a,b] Within columns, means with different letters are significantly different (P<0.05); SEM: Standard error of means

Table 10: Morphological indices of jejunum Ross 308 broilers in 42 d00 of age fed diets containing different concentrations of glibenclamide from 1-42 d of ageVilli/crypt depth

Glibenclamide (mg/kg)	Villi length (µm)	villi width (µm)	Crypt depth (µm)	
0	734	93	148	8.41
75	801	118	169	9.46
100	634	127	161	10.05

Table 11: Profile of breast fatty acids of broilers at 42nd day of age fed diets containing different levels of Glibenclamide powder

Glibenclamide (mg/kg)	Myristic Acid Methyl Ester C14:0 (%)	Palmitic Acid Methyl Ester C16:0 (%)	Palmitoleic Acid Methyl Ester C16:1c (%)	Stearic Acid Methyl Ester C18:0 (%)	Oleic Acid Methyl Ester C18:1n9c (%)	Linoleic Acid Methyl Ester C18:2n6c (%)	Linolenic Acid Methyl Ester C18:3n3 (%)	The ratio of saturated to unsaturated fatty acids
0	2.02	37.48	3.14	11.40	22.57	17.65	0.50	1.33
75	0.79	33.83	4.48	10.92	28.74	15.08	0.64	1.13
100	1.4	82.2	0.78	3.31	6.54	4.05	0.09	8.21

Their results suggest that the combined treatment of glibenclamide and caffeine can improve serum glucose control and result in

beneficial changes in serum triglyceride and HDL concentrations of diabetic rats, which is consistent with the results of the present study.

Immune system and relative weight of lymphatic organs

No differences were observed among treatments on humoral immune system function in response to SRBC antigen injection, except for 35 d which was highest for 100 mg/kg glibenclamide, and antibody titer against Newcastle virus and influenza (Table 7). In response to SRBC antigen injection, Newcastle and influenza titers increased at the end of the course.

Feeding glibenclamide did not increase the relative weight of the bursa of Fabricius or the weight of the spleen ($P \geq 0.05$). Metformin had a decreasing effect on the expression of RAGE. which implements the main pillar in the glycation cycle (Ishibashi *et al*. 2012) in the study that (Adeshara and Tupe. 2016) conducted on the anti-glycation and cell protection effects of metformin and the effect on erythrocyte and monocyte cells. Their results suggested that these compounds have a protective effect on the cell and can prevent the destruction caused by glycation. Adeshara and Tupe (2016) reported that metformin decreases in RAGE expression which is consistent with the results of Silvares *et al*. (2016). Liao (2018) reported that glibenclamide protects neonatal mice from developing bronchopulmonary dysplasia by inhibiting caspase-1 activation. The production of interleukin-1beta and the suppression of neutrophils and macrophages are related, so it can be stated that glibenclamide and other sulfonylurea drugs such as metformin can lead to strengthening of the immune system, which needs further study.

Microbial flora

Feeding 0 or 75 mg/kg glibenclamide supported lower numbers of *Escherichia coli* bacteria compared to other treatments ($P<0.05$); however, no differences were observed in the number of *lactobacillus acidophilus*. An increase in free radicals can lead to the oxidation and destruction of biological cells resulting in several disorders in the intestinal tissue (Ocak *et al*. 2008; Sahin *et al*. 2013). Diabetic rats treated with glibenclamide had lower *B. pseudomallei* populations in the liver and spleen, decreased neutrophil and macrophage infiltration in the lung, and decreased IL-1β levels compared with the control. Also, it showed in bronchoalveolar lavage fluid and lungs (Koh *et al*. 2013). Whether glibenclamide can have a similar protective role against other infections is still unclear and needs further study.

Intestinal morphology

Based on the results shown in Table 10, the highest villi length was observed with the consumption of 75 mg/kg glibenclamide. The depth of the crypt increased in the same dose of glibenclamide compared to the control group, but the ratio of villi length to the depth of the crypt decreased. Researchers believe that beneficial intestinal bacteria can play an important role in improving the function of the mucosal barrier, the maturity and health of the intestine, and the development of the lymphatic tissue, and their presence is necessary to improve the function of the intestinal barrier. On the other hand, the direct binding of Sur1 subunit to glibenclamide can be useful for protecting against damage related to inflammation in the central nervous system (CNS) and inhibiting Sur1-Trpm4 channels (Haj *et al*. 2004). Considering that the investigated substance can maintain the activity of beneficial intestinal bacteria and eliminate harmful bacteria, it is plausible that low concentrations of glibenclamide can maintain the health of the morphological indicators of the intestine. This needs further study.

Fatty acid profile of breast meat

The effect of treatments on the percentage of fatty acids in breast muscle tissue is outlined in Table 11. The results indicate that lower percentages of myristic and palmitic acid were observed when 75 mg/kg of glibenclamide was fed compared with 100 mg/kg; however, palmitoleic, steric, oleic,

linoleic, and linolenic acid percentages were lowest and the ratio of saturated to unsaturated fatty acids was highest when 100 kg/kg of glibenclamide was fed. Omega-3 unsaturated fatty acids have a double bond between carbon atoms number 3 and 4 and include the essential fatty acid linolenic acid. Linolenic acid is metabolized in the body and turns into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ansel Richard. 2005). Both EPA and DHA are finally converted into prostacyclin. Prostacyclin can cause blood clots and cause expansion of blood vessels. These fatty acids also prevent the production of thromboxane A2, which constricts blood vessels and collects platelets (Jan Quira *et al*. 2013). EPA and DHA are found in fish oil, especially fatty fish. Diabetic patients have less ability to convert linolenic acid to EPA and DHA (Shahabinejad 2006). Also, insulin in adipose tissue inhibits the activity of hormone-sensitive lipase enzyme, and as a result, not only the release of fatty acids, but also the secretion of glycerol decreases (Woodhavi 1999; Harper Harvold 2004), in addition, the storage of non-fatty acids multiple saturation of n-3 in breast muscle is more than thigh muscle. Eicosadienoic acid-11,14-cis, cis-8,11,14-eicosatrienoic acid and cis-11,14,17-eicosatrienoic acid are ideal as a standard for biological studies. These fatty acids are mainly found in small concentrations in animal tissues (Wang 2011; Huang *et al*. 2012) Eicosadienoic acid -11,14-cis by a delta-9 elongase enzyme from linoleic acid can be converted into di-homogamma-linolenic acids, arachidonic acid, siadonic acid and other unsaturated fatty acids. Eicosadienoic acid-11,14-cis is capable of modulating the metabolism of unsaturated fatty acids and is responsible for the response of macrophages to inflammatory stimuli. Together with other monounsaturated fatty acids, eicosadienoic acid-11,14-cis can inhibit leukotriene B4 binding to the membrane of neutrophils, which includes a part of these anti-inflammatory activities (Huang *et al*. 2011), that is, this compound protects the cell membrane and unsaturated fatty acids of the membrane against the oxidation of free radicals.

Finally we suggest effect of glibenclamide feeding in broiler study in human nutrition in future studies, since its residues should not transfer into human body.

Conclusion

In general, the results of this trial indicate that glibenclamide does not improved feed consumption, weight gain, conversion rate, total cost per kilogram of live chicken and production index in the rearing period. However, feeding low concentrations could reduce abdominal fat. Feeding low concentrations of glibenclamide led to a positive effect on blood factors, including triglycerides, cholesterol, VLDL, LDL, total protein. Populations of *Escherichia coli* were reduced in the intestine which appeared to improve villi length and crypt depth as well as the ratio of villi length to crypt depth compared to the control group. In comparison of saturated and unsaturated fatty acids, its low dose was able to reduce the ratio of saturated to unsaturated fatty acids. Therefore, according to the positive effects of glibenclamide on performance, carcass characteristics, blood parameters, immunity, intestinal flora, intestinal morphology and fatty acid profile, this feed additive can be used as an alternative to commercial antibiotics and a cheap growth promoter.

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چکیده

زمینه مطالعاتی: اثر سطوح بالای گلی بن کلامید بر عملکرد، ویژگی‌های لاشه، پارامترهای خونی، ایمنی، فلور میکروبی روده، مورفولوژی روده و پروفایل اسید چرب ماهیچه سینه در جوجه‌های گوشتی بررسی شد. **روش کار**: تعداد ۱۲۰ جوجه گوشتی یک روزه سویه تجاری راس ۳۰۸ به طور تصادفی در سه تیمار، هر کدام با ٤ تکرار شامل ۱۰ جوجه در هر پن توزیع شدند. تیمارهای آزمایشی شامل ۰، ۷۵ و ۱۰۰ میلی گرم بر کیلوگرم گلی بن کلامید بودند. **نتایج**: نتایج نشان می دهد که استفاده از غلظت های مختلف گلی‌بن‌کلامید در هیچ یک از دوره‌های پرورش تأثیر معنی‌داری بر عملکرد نداشت (P≥۰/۰۵)، اما بیشترین مصرف خوراک، افزایش وزن و بهترین ضریب تبدیل غذایی در دوره های ۱ تا ۱۰، ۲۲ تا ٤۲ و ۱ تا ٤۲ روز برای غلظت ۷۵ میلی گرم بر کیلوگرم گلی‌بن‌کلامید مشاهده شد (P≥۰/۰۵). کمترین هزینه تولید (P≥۰/۰۵) و بهترین شاخص تولید اروپایی (P≥۰/۰۵) با تغذیه ۷۵ میلی گرم بر کیلوگرم گلی‌بن‌کلامید مشاهده شد. تغذیه گلی‌بن‌کلامید بر خصوصیات لاشه تأثیری نداشت (P≥۰/۰۵)، بجز اینکه چربی شکمی (P≥۰/۰۵) با مصرف ۷۵ میلی گرم بر کیلوگرم گلی‌بن‌کلامید کاهش یافت. تغذیه ۷۵ میلی گرم بر کیلوگرم گلی‌بن‌کلامید باعث کاهش کلسترول، تری گلیسیرید، VLDL، LDL، پروتئین کل (P< ۰/۰۵)، کاهش تعداد *اشریشیا کلی*(P<0.05)، افزایش تعداد *لاکتوباسیلوس اسیدوفیلوس*(P≥0.05)، افزایش طول پرزها و عمق کریپت و کاهش نسبت اسیدهای چرب اشباع به غیر اشباع شد. **نتیجه-گیری نهایی**: نتایج این آزمایش نشان می‌دهد که تغذیه گلی‌بن‌کلامید بر ویژگی‌های لاشه، پارامترهای خونی، ایمنی، فلور روده، مورفولوژی روده و مشخصات اسیدهای چرب تأثیر مثبت دارد.

واژگان کلیدی: ایمنی، بیفیدوباکتریوم، پرز، ران، رشد، کلسترول