

EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS WITH OR WITHOUT GUANIDINO ACETIC ACID ON SOME ILEAL MORPHOLOGY AND BLOOD BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

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SUMMARY

A 2 x 3 factorial study examined some ileal morphology and blood biochemical parameters of broiler chickens fed diets containing graded levels of crude protein with or without guanidino acetic acid (GAA). Six diets containing two levels of crude protein (standard levels 23.01, 21.01, 19.04% or low levels 21.00, 19.03, 17.07%) in the starter, grower and finisher respectively supplemented with three levels (0.0, 0.06 or 0.12%) of GAA were used. Each treatment was randomly assigned to 6 replicates (10 male Hubbard broiler chicks per replicates). The results indicated that: Some ileal morphology (Chicken fed standard diets have significantly ($P < 0.05$) taller Villi height (1269 μm) than those fed low protein (-2% CP) diet (1140, μm). In addition, significant differences were observed in Villi height values between chickens fed (0.0% GAA) diets and those fed (0.06 and 0.12% GAA) diets (1125, 1271 and 1217 m) respectively, while, no significant differences were observed in villi width or crypt depth (m) among all treatments), and Some blood parameters (Dietary CP level (standard vs.-2 CP) and GAA supplementation level (0.0, 0.06 and 0.12%) had a significant effect on all blood plasma parameters, examined herein, including levels of total protein, albumin, globulin, A/G ratio, total lipid, triglyceride and cholesterol. On the other hand, chicks fed low protein diets (-2%CP) and (0.0% GAA) have significantly higher values of total plasma lipid, cholesterol and triglycerides)

Keywords: broiler chickens, guanidino acetic acid, ileal morphology and blood biochemical parameters.

INTRODUCTION

Several experiments with broiler chicks have shown that performance is adversely affected with low crude protein diets and investigated the potential reasons for decreasing performance (Laudadio et al., 2012).

On the other hand, there is a lack of report linking the effects of dietary nutrients especially that of protein to the improve of the gastrointestinal tract of poultry. According to Yamauchi (2002), the morphological changes of the intestinal villi in chicks are dependent on the presence of digested nutrients in the small intestinal lumen.

A slower turnover rate of the intestinal epithelium results in a lower maintenance requirement, which can finally lead to a higher growth rate or growth efficiency of the animal (Van Nevel et al., 2005). Thus, the changes in intestinal morphology may influence nutrient metabolizability and performance. In addition, a deeper crypt may indicate faster tissue turnover to permit renewal of the villus, which suggests that the host's intestinal response mechanism is trying to compensate for normal sloughing or atrophy of villi due to inflammation from pathogens and their toxins (Gao et al., 2008).

A diet containing a standard protein level with an increase in villus height in the ileum and duodenum has been previously reported in broilers fed a diet with a low CP level ($\geq 22\%$, Sterling et al., 2005), and has been explained by the development of the intestinal villi, thus increasing the efficiency of digestion and absorption. Because long villi are correlated with improved gut health (Baurhoo et al., 2007),

Wykes et al. (1996) reported that a low protein diet reduced the rate of protein synthesis in most body tissues, particularly the intestine. Protein is known as the most important factor in recovery of the intestine

after feed withdrawal (Maneewan and Yamauchi, 2005). It also plays a critical role as a dietary constituent for development of morphological traits (Incharoen et al., 2010).

Maneewan and Yamauchi (2003) reported that semipurified protein free diets were the slowest in promoting histological recovery after feed withdrawal, suggesting that protein is the most important factor in recovery after feed withdrawal. Buwjoom et al. (2010), assessing the histology of intestinal villi and epithelial cells in broilers using long-term feeding of low-CP diets, found that the ileal villi did not show specific alterations and that the chronic feeding of a low-CP diet induced a histological alteration. Moreover, this report suggests that long villi, large cell area, and many cells undergoing mitosis might be observed even in hyponutritional conditions to obtain deficient nutrients, not just in the hypernutritional conditions. This suggests that the hypotrophied histological alterations can indicate that a diet is not nutritionally well-balanced.

Moreover, Low et al., (2018) found that serum albumin (ALB) and total protein (TP) were linearly reduced by the dietary CP level reduction in both diets with and without protease ($p < 0.001$, $p = 0.042$, respectively) but was increasing for triglycerides (TG) and uric acid (UA). The level of TG increased linearly ($p < 0.001$) with the reduction of dietary CP in the protease supplemented group. Within the non-protease -supplemented group, the level of UA decreased quadratically ($p < 0.07$).

Guanidino acetic acid is synthesized in the liver and kidney from Arginine and Glycine and subsequently methylated by *S*-adenosylmethionine to form creatine, (Dilger et al., 2013), and finally, ATP donates a phosphorus moiety to form the high-energy compound, phosphocreatine (Meister, 1965). Thus, GAA may be important for poultry nutrition not only as a replacement for dietary Arginine, an essential nutrient, but also to support overall energy homeostasis of the bird.

Tossenberger et al., (2016) studied the effect of GAA levels 0, 0.6, or 6.0 g GAA per kg of feed from 1 to 35 days of age. They indicated that supplementation of 0.6 g/kg GAA did not improve weight gain (wg), although here a numerical improvement of about 0.07% units was observed. This is generally in contrast to other studies where addition of 0.6 g/kg GAA improved consistently BWG (Lemme et al., 2007a, 2007b, 2011; Michiels et al., 2012, Dilger et al., 2013), and breast meat yield (Lemme et al., 2007a, 2007b, 2010a; Heger et al., 2014) in broilers and turkeys.

Information on the effect of GAA supplement on intestinal morphology in poultry is limited. The vast majority of information has addressed the role of arginine on gut morphology and function. Murakami et al. (2014) reported benefits of arginine supplement on morphometry of the duodenum mucosa in broiler chickens.

Khajali et al. (2014) reported increased villus height, width, and absorptive surface area in the jejunum as a consequence of arginine supplementation (10 g/kg). Increase in villus height increases total luminal villus absorptive area and subsequently results in satisfactory digestive enzyme action and higher transport of nutrients at the villus surface (Tufarelli et al., 2010).

Ahmadipour et al. (2018) found that the villus height, width and absorptive surface area in duodenum, jejunum, and ileum sections were significantly ($P < 0.05$) improved at GAA supplementation above 0.5g/kg. However, the crypt depth showed a significant decrease in all parts of the small intestine compared to the control.

Khakran et al. (2017) fed Hy-Line W-36 laying hens levels of GAA (0, 0.057, 0.114 and 0.171%), and found that average blood biochemical parameters (Triglycerides, HD, LDL, AST and ALT) of chickens at 29 weeks of age did not differ among the treatments.

Therefore, this study was conducted to determine the best levels of GAA in standard or low protein diets to achieve their sparing effect on ileal morphology and blood biochemical parameters of broiler chickens.

MATERIAL AND METHODS

This study was carried out at poultry experimental and research station at Shalakan, Faculty of Agriculture, Ain Shams University, in order to evaluate the differences in histology of intestinal villi and blood biochemical parameters of broiler chickens fed on six starter, grower and finisher diets containing the studied two protein and three (GAA) levels.

Birds and management:

Total of 360 one day old of male Hubbard broiler chicks were used, weighed and distributed into six experimental group (60 chick each) in 2 x 3 factorial completely randomized design (two protein and three GAA) levels. Each treatment group was consisted of six replicates of 10 chicks each. Chicks were reared in electrically heated batteries under similar hygienic, environmental and managerial conditions.

All chicks were vaccinated against the common viral diseases (NDV, IBDV) at the recommended periods. Feed and water were supplied ad-libitum and a constant (22 L: 2D) light period was provided during the experimental periods (1-35) day of age.

Experimental diets:

Six starter, grower and finisher diets were formulated to contain the studied protein and GAA levels. Three periodical diets were formulated in the experiments includes, starter (1-14), grower (15-28) and finisher (29-35) days of age. The standard diets (SD, T₁, T₂ and T₃) were formulated according to the manual guide of Hubbard broiler chicks, while tested diets (TD, T₄, T₅ and T₆) were represent all requirements of birds except for crude protein which was reduced by about 2% of (starter grower or finisher) diets and each diets from (SD or TD) supplemented which 0.0, 0.06 or 0.12% GAA respectively. The dietary treatments were as follows:

T₁ standard diets (SD) + 0.0% (GAA)

T₂ standard diets (SD) + 0.06% (GAA)

T₃ standard diets (SD) + 0.12% (GAA)

T₄ Tested diets (-2% CP) + 0.0% (GAA)

T₅ Tested diets (-2% CP) + 0.06% (GAA)

T₆ Tested diets (-2% CP)+ 0.12% (GAA)

- The standard and tested diets are shown in Table (1)

- GAA, CreAmino, contain 94% guanido acetic acid and purchased from Evonik Industries

Measurements

Blood sampling

At the end of experimental period (35 days of age), five chickens were randomly taken from each dietary treatments and slaughtered. Blood samples were taken in dry clean centrifugation from the slaughtered birds and plasma were separated by centrifugation at 3000 rpm for 15 minutes and assigned for subsequent determination.

Plasma samples were harvested after centrifugation of the clotted blood, stored at -20°C in the deep freezer until time of chemical determinations.

Some blood plasma components

Biochemical analysis of blood plasma was conducted in Cairo University Research Park (CURP), Faculty of Agriculture. Quantitative determination of blood total protein, albumin, globulin, total lipids, cholesterol and triglycerides were calorimetrically determined using commercial diagnosing kits (Produced by Bio-diagnostic Company, Egypt).

Some ileal morphological

At the end of the experiment, representative tissue samples from ileum were taken to study the histological changes associated with the experimental treatments.

Three birds per treatment having the mean body weight of the group were used from each treatment for histological observations the tissue samples from the ileum of approximately midway between Meckel's diverticulum and the ileocaecal junction. Segments were flushed with saline solutions (0.9% NaCl) to remove contents and were fixed in neutral buffered formalin solution for histology; samples were dehydrated, cleared The rotary type microtome was used for cutting the paraffin sections.

All sections were examined under electric microscope provided with computerized camera. Villus height, Villus width and crypt depth were determined and calculated. The values were measured with an oculometer

at a magnification of 5x under a light microscope fitted with the stage micrometer and using an image analyzer (Leica Microsystems Co., Ltd, Germany) connected to a light microscope.

Table (1): Composition and calculated chemical analyses of experimental diets

Ingredient %	Standard diet*			-2% (CP) diet **		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Yellow corn	51.55	57.23	62.59	56.91	62.61	67.92
Soybean meal (44%)	35	29.79	24.70	30.20	24.96	19.90
Corn gluten meal (62%)	5.20	4.90	4.60	4.70	4.40	4.10
Soy oil	3.5	4.00	4.25	3.00	3.50	3.75
Limestone	1.35	1.10	1.08	1.35	1.12	1.10
Di-calcium phosphate	1.90	1.68	1.55	1.95	1.71	1.60
Premix***	0.30	0.30	0.30	0.30	0.30	0.30
Salt (NaCl)	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.31	0.24	0.21	0.37	0.30	0.26
Lysine HCl	0.32	0.25	0.23	0.44	0.38	0.35
L-Arginine	0.07	0.05	0.04	0.21	0.18	0.19
L-Threonine	0.10	0.06	0.05	0.17	0.14	0.13
Total	100	100	100	100	100	100
Calculated analysis ****						
ME (kcal/ kg)	3040	3151	3230	3045	3157	3235
CP%	23.01	21.01	19.04	21	19.03	17.07
Calorie / protein ratio (C/P)	132	150	170	145	166	190
Calcium %	1.05	0.90	0.85	1.05	0.90	0.85
Available Phosphorous %	0.50	0.45	0.42	0.50	0.45	0.42
Methionine %	0.69	0.60	0.54	0.73	0.63	0.56
Methionine + cysteine %	1.08	0.99	0.91	1.08	0.96	0.86
Lysine	1.44	1.29	1.16	1.44	1.29	1.16
Arginine	1.46	1.29	1.14	1.46	1.28	1.15
Threonine	0.95	0.84	0.75	0.95	0.84	0.75
Tryptophan	0.31	0.27	0.24	0.27	0.24	0.20

* Standard diets: represent all requirements of birds according to standards requirement guide.

** -2% (CP) diets: represent all requirements of birds according to requirements, except for crude protein which was reduced by about 2% of (starter, grower or finisher) diets.

*** Composition of each 3 kg of vitamin and minerals premix contain: 15000000 I.U VIT. A, 5000000IU Vit. D3, 50000 mg. VIT. E, 3000 mg. VIT. K3, 3000 mg. VIT. B1, 8000 mg. VIT. B2, 4000 mg. VIT. B6, 20 mg. VIT. B12, 15000 mg. Pantothenic acid, 60000 mg. Niacin, 1500 mg. Folic acid, 200 mg. Biotin, 200000 mg VIT C, 700 gm. Choline chloride, 80 gm. Mn, 80 gm. Zn, 60 gm. Iron, 10 gm. Cu, 1 gm. Iodine, and 0.2 gm. Selenium, where CaCo3 was taken as a carrier up to 3 kg, the inclusion rate was 3 kg premix / Ton feed

**** Calculated analyses of the experimental diets were done according to (NRC, 1994).

Statistical analysis

Data obtained were analyzed using the general linear models procedure of SAS software (SAS Institute, 2001). When differences among means were found, means were separated using Duncan's (Duncan's 1955) multiple ranges test at (P<0.05). The statistical model was:

$$Y_{ijk} = \mu + D_i + E_j + D * E_{ij} + e_{ijk}$$

Where: Y_{ijk} = the effect of the observation, μ = The overall mean, D_i = The effect of the diets, e_j = The effect of the feed additives, $D * E_{ij}$ = The interaction between diets and feed additives, e_{ijk} = The experimental error

RESULTS AND DISCUSSION

Effect of different dietary protein levels with or without guanidino acetic acid on some ileal morphology measurements of broiler chicks.

Results of some ileal morphology values (Villi height and width, μm) and crypt depth (μm) are presented in Table (2) and Figure (1) and demonstrated that, chicken fed standard diets have significantly ($P < 0.05$) longer Villi height (1269, μm) than those fed low protein (-2% CP) diet (1140, μm). In addition, significant differences were observed in Villi height values between chickens fed (0.0% GAA) diets and those fed (0.06 and 0.12% GAA) diets (1125, 1271 and 1217 μm) respectively.

Table (2): Effect of different dietary treatments on some ileal morphology measurements, of broiler chickens

Item	Crude protein (CP)	GAA * %			Overall	Significant			
		0.00	0.06	0.12		MSE	CP	GAA	CP*GAA
	Standard diets**	1249	1324	1234	1269a				
Villi height (μm)#	-2% (CP) diets***	1002	1219	1200	1140b	115	*	*	*
	overall	1125b	1271a	1217ab					
	Standard diets**	229	332	261	274				
Villi width (μm)##	-2% (CP) diets***	212	230	261	234	90	NS	NS	NS
	overall	220	281	261					
	Standard diets**	142	135	187	155				
Crypt depth (μm)	-2% (CP) diets***	170	137	139	148	36	NS	NS	NS
	overall	156	136	163					

* GAA: Guanidino acetic acid.

** Standard diets: represent all requirements of birds according to standards requirement guide.

*** -2% CP diets: represent all requirements of birds according to standards requirement guide, except for crude protein which was reduced by about 2% of (starter, grower or finisher) diets.

Tip to the bottom of villus

##Width at villus base.

a,b: Means in the same row or column with the same letters are not significantly different. MSE: Mean standard error
NS: Non-significant, *: ($P \leq 0.05$) **: ($P \leq 0.01$)

As shown in Figure (1) no significant differences were observed in villi width or crypt depth (μm) between all treatments. Numerically, the chickens fed standard diets had the highest villi width (274 μm) and crypt depth (155 μm) compared with chickens that fed low protein diets, being (234 and 148 μm), respectively.

Also, villi width and crypt depth (μm) for the chickens fed the diets containing all levels of GAA showed insignificant differences. However, the chickens fed diet containing (0.06% GAA) had the highest villi width (281 μm) and chickens fed 0.12% GAA had the highest crypt depth (163 μm) compared with other dietary treatments.

Obtained results agree with those reported by Incharoen et al. (2012), Laudadio et al. (2012) and Low et al. (2018) who all reported that the dietary CP level influenced the morphology of the mucosa in the small intestine and the villus height in duodenum villus area decreased in the low CP group. In the same order Emami et al. (2016) and Ahmadipour et al. (2018) found that GAA supplementation to broiler diets villus surface area compared to control group.

Effect of different dietary protein levels with or without guanidine acetic acid on some blood parameters of chicks.

Data of blood parameters for 5 weeks age broiler chicks fed the experimental diets containing different CP and/or supplemented with GAA are presented in Table (3) and Figures (2). It is evident that dietary CP level (standard vs.-2 CP) or GAA supplementation level (0.0, 0.06 and 0.12%) had a significant effect on all blood plasma parameters, examined herein, including levels of total protein, albumin, globulin, A/G ratio, total lipid, triglyceride and cholesterol.

Table (3): Effect of different dietary treatments on some blood plasma components of broiler chickens.

Item		Crude protein (CP)			overall	Significant			
		0.00	0.06	0.12		MSE	CP	GAA	CP*GAA
Total protein (g/dl)	Standard diets**	2.50	3.84	3.53	3.29 ^a				
	-2% (CP) diets***	2.31	3.47	2.81	2.86 ^b	0.12	**	**	**
	overall	2.41 ^c	3.66 ^a	3.17 ^b					
Albumin (g/dl)	Standard diets**	1.15	1.25	1.30	1.23 ^a				
	-2% (CP) diets***	1.10	1.23	1.10	1.14 ^b	0.07	*	*	*
	overall	1.13 ^b	1.24 ^a	1.2 ^{ab}					
Globulin (g/dl)	Standard diets**	1.35	2.59	2.23	2.06 ^a				
	-2% (CP) diets***	1.21	2.24	1.71	1.72 ^b	0.08	**	**	**
	overall	1.28 ^c	2.42 ^a	1.97 ^b					
A/G ratio	Standard diets**	0.85	0.48	0.58	0.64 ^b				
	-2% (CP) diets***	0.91	0.55	0.65	0.70 ^a	0.04	**	**	NS
	overall	0.88 ^a	0.52 ^c	0.62 ^b					
Total lipid (mg/dl)	Standard diets**	1506.77	1142.24	1131.51	1260.17 ^b				
	-2% (CP) diets***	1908.98	1155.86	1412.75	1492.53 ^a	26.19	**	**	**
	overall	1707.88 ^a	1149.05 ^c	1272.13 ^b					
Triglyceride (mg/dl)	Standard diets**	216.00	125.33	132.00	157.78 ^b				
	-2% (CP) diets***	267.33	144.67	146.00	186.00 ^a	15.85	**	**	NS
	overall	241.67 ^a	135.00 ^b	139.00 ^b					
Cholesterol (mg/dl)	Standard diets**	154.49	110.27	112.49	125.75 ^b				
	-2% (CP) diets***	166.88	113.67	126.64	135.73 ^a	5.08	**	**	NS
	overall	160.69 ^a	111.97 ^c	119.56 ^b					

* GAA: Guanidino acetic acid.

** Standard diets: represent all requirements of birds according to standards requirement guide.

*** -2% CP diets: represent all requirements of birds according to standards requirement guide, except for crude protein which was reduced by about 2% of (starter, grower or finisher) diets.

a,b: Means in the same row or column with the same letters are not significantly different. MSE: Mean standard error
NS: Non-significant, *: ($P \leq 0.05$) **: ($P \leq 0.01$)

Significantly, best values were found in broiler chicks that fed, standard diets and diets supplemented with 0.06% GAA than other treatments.

Dietary CP and GAA had significant effect on total protein ($P \leq 0.05$). The highest values was noticed when diet supplemented with 0.06% GAA than 0.12% and control. But standard diets had the highest value (3.2) than (-2%) CP diet. These results also evident in albumin and globulin parameters. These results were logically, because decrease CP is effect on the protein metabolism and in birds body. Regarding to globulin and A/G ratio, the results elucidated the GAA improve the bird's immunity especially 0.06% than 0.12% and control.

According to lipid metabolism the results illustrated that GAA supplementation had decreased total lipid, triglyceride and cholesterol concentration than control especially 0.06%. That mean, these treatments had decreased the lipid metabolism and subsequently improved the bird's health. On the other hand, decreased CP in the diet had increased the triglyceride, and cholesterol concentrations in the body of the bird. These results normally logic, because any defect in the diet had reflected on the some substances metabolism in the bird's body.

On the other hand, chicks fed low protein diets (-2%CP) and (0.0% GAA) have significantly higher values of total plasma lipid, cholesterol and triglycerides, while, chicks fed standard diets and diets supplemented with GAA (0.06 and 0.12%) have significantly lowest values of these measurements of blood. Similarly, Kamran et al. (2004) studied the effect of lowering dietary crude protein (CP) with optimum limiting amino acids levels on blood parameters of broilers from 1 to 35 days of age and found that plasma uric acid concentration decreased and triglycerides concentration increased significantly by lowering the dietary CP content.

Gadelrab (2014) and Abd El-Hady (2012) who found no significant effects of protein programs (protein levels) and supplementing amino acid or with ME level on blood plasma constituents including levels of total protein, albumin, triglycerides, activities of transaminases (AST and ALT), total lipids and cholesterol.

CONCLUSION

From the present study, it could be concluded that supplemental low protein broiler diets (-2% CP) with 0.06% (GAA) diet would have a positive effects on some ileal morphology and blood biochemical parameters.

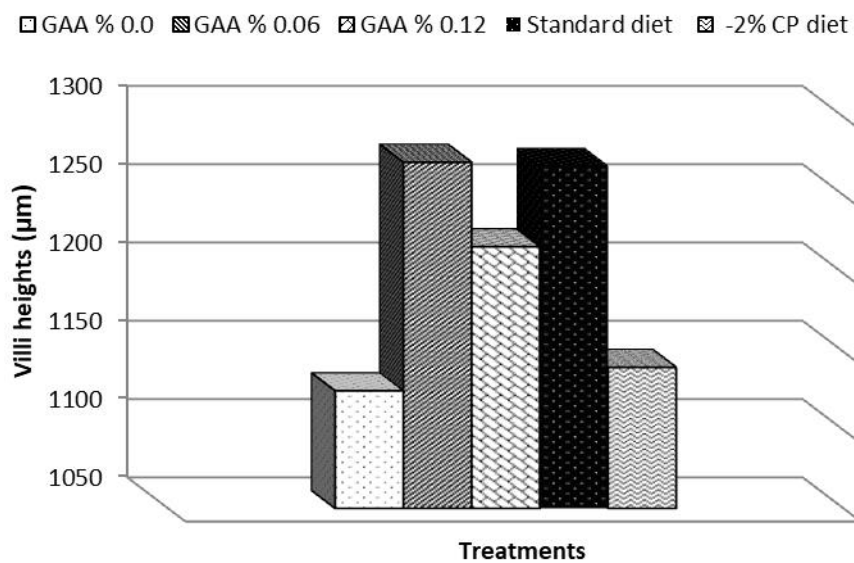
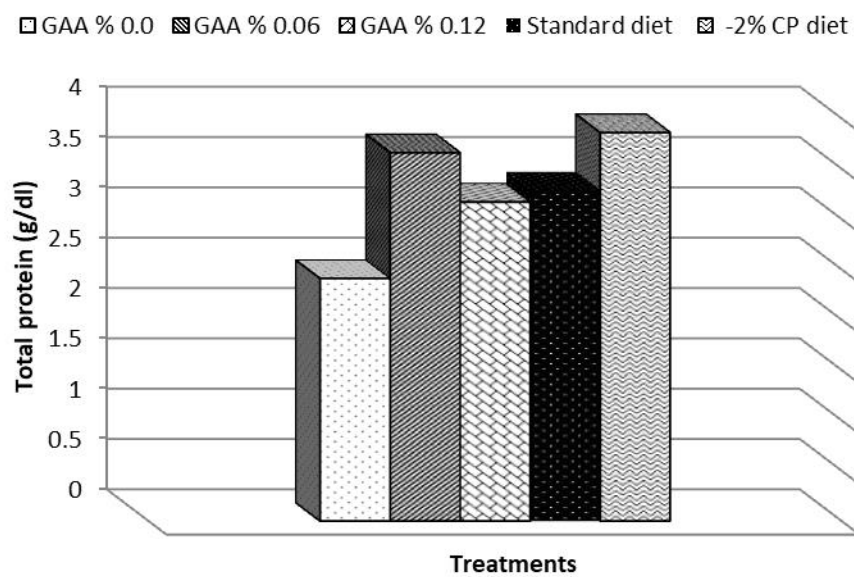


Fig. (1): Effect of different dietary treatments on some villi height of broiler chickens



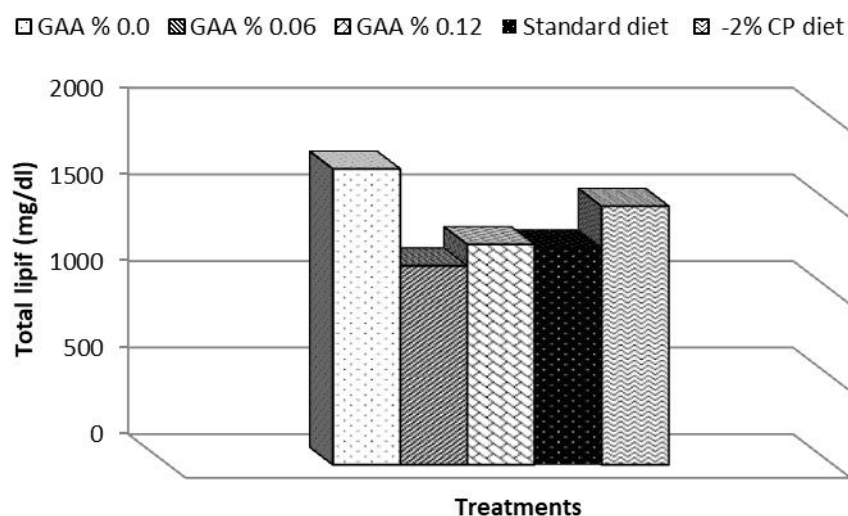


Fig. (2): Effect of different dietary treatments on some blood plasma components of broiler chickens

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تأثير المستويات المختلفة من بروتين العليقة مع أو بدون إضافة جوانيدو أستيك أسيد على بعض الصفات المورفولوجية للأمعاء وقياسات الدم لبدارى التسمين

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أجريت تجربة عاملية (2 × 3) لدراسة بعض الصفات المورفولوجية للأمعاء وقياسات الدم لبدارى التسمين المغذاة على علائق تحتوي على مستويات مختلفة من البروتين مع أو بدون إضافة (جوانيدو أستيك أسيد). استخدم 6 علائق أحتوت على مستويين من البروتين (مستوى قياس 23.01، 21.1، 19.04% أو مستوى منخفض 21.00، 19.03، 17.07%) في علائق البادى والنامي والناهي مضاف إليها 3 مستويات (0.0، 0.06، 0.12%) من جوانيدو أستيك أسيد. كل معاملة أحتوت على 6 مكررات بكل مكرر 10 كتاكيت ذكور هبرد عمر يوم.

أهم النتائج المتحصل عليها:

* بعض الصفات المورفولوجية للأمعاء

- سجلت الطيور المغذاة على العلائق القياسية أعلى قيم لطول الخملات بالمقارنة بتلك المغذاة على عليقة منخفضة في البروتين (-2%) 1269 مقارنة بـ 1140 ميكرون.

- سجلت الطيور المغذاة على عليقة بدون إضافة جوانيدو أستيك أسيد أقل القيم لطول الخملات بالمقارنة بتلك المضاف إليها بمعدل (0.06% أو 0.12%) فكانت (1125 ضد 1271 أو 1217 ميكرون)

* بعض قياسات بلازما الدم

- تأثرت قياسات الدم معنوياً بمستوى بروتين العليقة وكذلك بمستوى إضافة جوانيدو أستيك أسيد (البروتين الكلي والألبومين وجليوبولين والدهون الكلية والدهون الثلاثية والكوليسترول).

نستخلص من هذه الدراسة إمكانية تخفيض محتوى علائق بدارى التسمين (بادى والنامي والناهي) 2% بروتين خام مع إضافة 0.06% علف جوانيدو أستيك أسيد للحصول على أفضل نتائج للصفات المورفولوجية للأمعاء وقياسات الدم.