



Indices of Serum Biochemical of Broiler Fed Diet Containing Local Broiler Premix as an Alternative to Commercial Broiler Premix

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ABSTRACT

It is essential to examine and harness the potential of local broiler premixes as alternatives to expensive commercial premixes, while ensuring that would not have any harmful impact on broiler health. A total of 120 broiler chicks (Ross 308) were used in a three-week study to examine the impact of different levels of local broiler premix (LBP) as a substitute for vitamin-mineral premix on the serum biochemical profile of broiler chickens. The chicks were assigned to four treatments, with each treatment consisting of thirty birds. The experiment group contained three replicates each with ten birds per replicate, following a Completely Randomized Design. The supplementation levels of the local broiler premix were as follows: 2.50% for commercial broiler premix (CBP1), 1.85% for LBP2, 2.25% for LBP3, and 2.50% for LBP4. The results indicate that the supplementation of LBP to broiler diets did not result in any significant changes in the enzyme activity of Glu, TP, Alb, and Glo. The TC concentration in the group following the LBP2 diet was significantly higher compared to the concentration in the CBP1 group. A significant positive correlation ($P < 0.01$) was seen between the following pairs: Glu and Alb, TG and VLDL, TG and ALT, VLDL and ALT, as well as TP and Alb. Hence, the use of local broiler premix did not have any adverse impact on the blood biochemical profile of starter broiler chickens, indicating its suitability as a substitute for commercial vitamin-mineral premix.

KEY WORDS:

Liver enzymes, Alternative feed, Premix, Broiler, Enzyme activity

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الصفات الكيموحيوية لدم فروج اللحم سلالة Ross 308 المغذى على عليقة حاوية على خليط فيتامينات ومعادن محلي الصنع كبديل للبريمكس التجاري قاسم عاجل شناعة الزياي¹

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الخلاصة

من المهم دراسة واختبار علائق محلية الصنع خاصة بتغذية فروج اللحم تمتاز بأسعارها المناسبة كبديل عن العلائق الجاهزة التي تتميز بارتفاع أسعارها وكلفتها العالية مع الأخذ بنظر الاعتبار الأداء العالي لهذه العلائق وأثرها على الحالة الصحية لفروج اللحم. استخدمت في هذه التجربة 120 طير فروج اللحم سلالة Ross 308 بعمر يوم واحد لدراسة تأثير إضافة مستويات مختلفة من البريمكس المحلي الصنع كمصدر للفيتامينات والأملاح في علائق البادئ لفروج اللحم في الصفات الكيموحيوية لبلازما الدم. قسمت الأفراخ بشكل عشوائي إلى أربعة معاملات، إذ احتوت كل معاملة على 30 طير، وكل معاملة تحتوي على 3 مكررات بواقع 10 طير لكل مكرر. اضيف خليط الفيتامينات والمعادن بالمستويات التالية: 2.5 % للبريمكس التجاري (CBP1)، بينما كانت المعاملات التي غذيت على خليط الفيتامينات والمعادن المحلي الصنع 1.85% للمعاملة (LBP2)، 2.25% للمعاملة LBP3 و 2.5% للمعاملة LBP4 على التوالي. بينت النتائج بان إضافة خليط الفيتامينات والمعادن المحلي الصنع الى عليقة فروج اللحم لم تؤثر معنوياً في تراكيز كل من الكلوكرز، البروتين الكلي، الألبومين والكلوبيولين في بلازما الدم. بينما تشير النتائج الى وجود ارتفاع معنوي ($P < 0.01$) في التركيز الكلي للكلوستيرول للمجموعة LBP2 عند المقارنة مع المجموعة CBP1، كما تشير النتائج الى وجود ارتباط معنوي موجب ($P < 0.01$) وبين كل من الكلوكرز والألبومين، الكليسيريدات الثلاثية و البروتينات الدهنية واطنة الكثافة VLDL، الكليسيريدات الثلاثية و تركيز انزيم ALT وما بين تركيز البروتين الكلي وتركيز الألبومين لبلازما الدم. ويتضح بشكل عام بان إضافة البريمكس المحلي الصنع كمصدر للفيتامينات والأملاح في علائق البادئ لتغذية فروج اللحم لم يكن له تأثير سلبي في المؤشرات الكيموحيوية للدم مما يشير الى إمكانية استخدامه كبديل عن البريمكس التجاري. الكلمات المفتاحية: انزيمات الكبد، الاعلاف البديلة، بريمكس، فروج اللحم، النشاط الانزيمي.

INTRODUCTION

The rising prices of food products in Iraq have also affected the costs of animal feeds and feed ingredients, resulting in decreased profitability for farmers. The increasing costs of feeds do not relate proportionally to the market worth of livestock products such as broilers. This demands an immediate requirement to investigate a feed formulation that will maintain the poultry businesses at a lower cost of production. Researchers have thoroughly attempted to find more effective methods (Castro *et al.*, 2023; Mohammed , and Hamad, 2024; Sani *et al.*, 2024) to decrease the expense of poultry feed, which accounts for 70-80% of the overall production cost in the poultry industry (Jha *et al.*, 2019). Hence, possessing sufficient understanding of poultry nutrition and, naturally, micro-nutrients (such as vitamins and minerals) found in alternative feed ingredients is essential for effective ration formulation (Al-Obaidi *et al.*, 2022; Belim *et al.*, 2021).

The inclusion of a vitamin-mineral premix in broiler chicken feed is crucial because the digestive system of broilers cannot produce a sufficient amount of vitamins and minerals (Ogunwole and Mosuro, 2020). Consequently, these items are costly and occasionally difficult to find, highlighting the need to find practical substitutes to tackle the economic difficulties encountered by farmers in rural regions of developing countries. However, a study investigated excluding vitamin and trace mineral supplementation from broiler diets between 35 and 42 days of age led to lower weight increases among several broiler strains (Mochamat *et al.*, 2017). On the other hand, vitamin-mineral premixes can help broiler chickens grow and maintain health. If vitamin-mineral premixes are not appropriately balanced, though, it may have significant health consequences. Several health disorders such as, toxicity and metabolic abnormalities may follow from over-supplementation (Ali

et al., 2019). For example, excessively high levels of vitamins A and D could lead to hypervitaminosis, which damages the liver, weakens the bones, and compromises kidney performance (Rahman *et al.*, 2012).

Likewise, large concentrations of several minerals, like calcium and phosphorous, might upset the absorption of other required nutrients causing poor growth and bone disorders. Therefore, in broiler diets, it is imperative to identify other feeds that can provide vitamins and trace minerals combined (Shaman and Mohammed, 2023). This suggested research is justified by the possible advantages of including locally plentiful feed products in broiler diets. Using these components instead of commercial premixes could help to drastically lower feed expenses. Furthermore, supplementing these indigenous elements to broiler chicken diets can improve their welfare and provide a sustainable and reasonably priced substitute for chicken nutrition. Hence, this study aimed to evaluate the enzyme activity of broiler chickens fed diets substituting locally produced premix for commercial vitamin-mineral premix.

MATERIALS AND METHODS

The study was conducted following the guidelines established by the Animal Care and Use Committee of the College of Agriculture at the University of Anbar (Approval No. 162/2022).

A total of 120 broiler chicks (Ross 308) were purchased from a commercial hatchery. On the initial day, the weight of the chicks was documented and distributed arbitrarily among 12 floor pens that have dimensions of 1.2 m by 1.2 m. The experimental diets consisted of the same feedstuff. The local broiler premix (LBP), which contained abundant sources of vitamins and minerals, was obtained from the market and thoroughly mixed using an improvised mixer.

The local premix was made and utilized to substitute the commercial broiler premix (CBP) in the diet at different levels. The CBP1 included the basal diet along with 2.5% of commercial premix. LBP2 included the basal diet along with 1.85% of local premix. LBP3 included the basal diet along with 2.25% of local premix. LBP4 included the basal diet along with 2.50% of local premix. Feed and water were provided freely and without restriction throughout the entire duration of the experiment. The premix that was previously prepared was mixed with other feed ingredients that were utilized to create the experimental diets, as shown in Table 1. The diet's chemical compositions were determined following procedures (AOAC, 1990).

Table 1. Ingredients (%) and chemical compositions of experimental diets.

Item	CBP1	LBP2	LBP3	LBP4
Corn	30.500	37.750	39.780	39.780
Wheat	13.00	5.80	2.47	1.17
Wheat Flour	10.00	8.00	8.00	8.00
Soybean meal (46%)	38.00	40.30	41.00	41.25
Corn oil	3.90	4.00	4.20	5.00
CaCO ₃	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.50	1.70	1.70	1.70
Salt	0.10	0.10	0.10	0.10
Commercial premix	2.50	0.00	0.00	0.00
Local broiler premix	0.00	1.85	2.25	2.50
Total	100.00	100.00	100.00	100.00
Chemical components (%)				
Crude protein	23.9	22.07	22.12	24.6
Ether extract	2.04	2.10	2.10	2.07
Crude fiber	4.02	4.07	4.06	4.04
Calcium	0.96	0.79	0.83	0.86
Phosphate	0.86	0.85	0.89	0.92
Lysine	1.39	1.46	1.52	1.56
Methionine	0.59	0.54	0.58	0.60
Threonine	0.40	0.55	0.59	0.61
Cysteine	0.61	0.37	0.37	0.37
Cysteine + methionine	1.20	0.91	0.95	0.97
Metabolizable energy (kcal)	2836	2958.70	2956.56	2930

Commercial premix consists: crude protein 10%, lysine 1.6%, methionine 6.40%, methionine + cysteine 6.05%, threonine 0.160%, choline chloride 12000 mg/kg, arginine 0.3%, valine 0.20%, isoleucine 0.13%, tryptophan 0.05%, fat 2.65%, ash 78.09, calcium 20.40%, phosphorus 4.90%, sodium 5%, metabolizable energy 3897 kcal/kg, zinc 2400 mg/kg, selenium 10 mg/kg. Vit. A 400000 mg/kg, Vit. D3 160000mg/kg, Vit. E1200 mg/kg, Vit. B1120 mg/kg, Vit. B2 280 mg/kg, Vit. B6 160 mg/kg, Vit. 121400 mg/kg, biotin 4 mg/kg, niacin1600 mg/kg, folic acid 40 mg/kg, Vit. K3 100 mg/kg, dicalcium pantothenate 600 mg/kg, choline10411 mg/kg, iron 2000 mg/kg, copper 400 mg/kg, manganese 3200 mg/kg, iodine 40 mg/kg, crude fiber 0.25%.

Local broiler premix supplied the following per kilogram of diet which consists: Premix Vitamins 1.5, Mineral premix 1.5, Methionine (99%) 2, Lysine (98.5%) 2.5, Threonine (98.5%) 2Heptaenzyme 0.5, Profits 0.2, Antitoxin 1, Choline chloride (60%) 1, Monocalcium (22%) 7, Anticoccidia 0.5, Optivid 0.5.

On the 21st day, blood samples were obtained from 9 birds in each treatment group (3 from each replicate) by puncturing the heart using vacutainer tubes (BD Bioscience, Franklin Lakes, NJ, USA) for serum clinical chemistry analysis. Following the collection of blood, the samples were stored at ambient temperature. Subsequently, the serum was isolated and subjected to centrifugation at a speed of 2500 rpm for 15 minutes using a centrifuge machine. Blood chemistry was analyzed using a clinical chemistry analyzer manufactured by Chiron Corporation, located in San Jose, CA, USA. The levels of glucose (Glu), total cholesterol (TC), total triglyceride (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), alanine transaminase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), and globulin (Glo) in the serum were measured.

Statistical analysis:

The data were analyzed using ANOVA as a completely randomized design with the GLM procedure of SAS software (SAS version 9.2., SAS Institute, Inc., Cary, NC). Additionally, Pearson correlation coefficients have been calculated for the biochemical blood characteristics, specifically for peptide and steroid chemical structures. Differences were assessed using Duncan's Multiple Range test with a significance level of 0.05.

RESULT AND DISCUSSION

The proximate analysis of the four experimental rations is presented in Table 1. The findings suggest that diets with PBL had comparable levels of metabolizable energy (ME), crude protein (CP), ether extract (EE), and methionine content compared to the control diet (CBP1). Although the proximate analysis of experimental rations indicated a low level of cysteine in LBP 2, LBP3, and LBP4, the approximate analysis of rations revealed that lysine and threonine were considerably higher in LBP 2, LBP3, and LBP4, respectively, compared to CBP1. The elevated concentrations of lysine and threonine in the rations of LBP 2, LBP3, and LBP4 suggest that both treatments contained sufficient nutrients, including a combination of vitamins and minerals from the local broiler premix, which may affect the consumption of crucial necessary amino acids. The bioavailability of minerals and vitamins in broiler chicken diets enhances feed consumption. Severe lack of threonine, but not lysine, leads to an increase in the expression of appetite-stimulating hypothalamic neuropeptides and a decrease in the expression of appetite-suppressing neuropeptides. This could be the reason for the higher feed intake (Abdelqader *et al.*, 2023; Wessels, 2022).

Table 2 presents the effect of experimental diets on the biochemical parameters of broiler serum. There were no significant variations observed in the enzyme activity of Glu, TP, Alb, and Glo when LBP was supplemented with broiler diets. There was no significant difference in the levels of AST, ALT, or the ratio of AST to ALT. The average values obtained in this study remain between the range of serum proteins generated in the liver to control blood volume through the colloidal osmotic effect, maintain blood pH, transfer hormones and drugs, help blood clotting, catalyze enzymatic reactions, control hormones, and defend the body against foreign substances (Tatar *et al.*, 2023). Combined, the remaining Glo and Alb levels produce the TP content. An enzyme presents in the liver; Alanine aminotransferase is secreted upon liver injury. This investigation showed that the liver was not harmed by these supplements since the ALT levels in the local broiler premix and commercial premix were not changed.

Table 2. The enzyme activity of structured peptides influenced different levels of experimental diets.

Variables	Glu (mmol/L)	AST (U/L)	ALT (U/L)	TP (g/dL)	Alb (g/dL)	Glo (g/dL)	AST:ALT (%)
CBP1	230.00	202.75	6.00	3.83	1.75	2.10	37.97
LBP2	249.50	193.50	6.50	3.93	1.83	2.08	37.40
LBP3	252.00	195.00	6.25	4.00	1.93	2.07	38.72
LBP4	250.50	171.50	5.50	3.87	1.85	2.02	32.65
SEM	4.117	7.255	0.558	0.0359	0.036	0.029	4.250
P-value	0.189	0.505	0.946	0.385	0.434	0.870	0.966

Glu: glucose, AST aspartate aminotransferase, ALT alanine transaminase, TP: total protein, ALB: albumin, and Glo: globulin. CBP1 (basal diet + 2.5% of commercial premix). LBP2 (basal diet + 1.85% of local premix). LBP3 (basal diet + 2.25% of local premix). LBP4 (basal diet + 2.50% of local premix).

This implies that the supplements could have hepato-protecting qualities, thereby enhancing liver condition (Liu *et al.*, 2022). This result corresponds with the research carried out by Alqhtani *et al.*, 2022, which showed that dietary factors had no influence on the comparable degrees of blood TP, Alb, Glo, ALT, and AST among all groups. Nevertheless, the inclusion of different levels of local broiler premix in the diet may lead to a maintenance in the performance of broiler chickens within the health range. Probably, the reason for this result can be attributed to the fact that the chickens in this study had reduced levels of stress when located in small-scale and intense care conditions (Riber *et al.*, 2018).

No statistically significant differences ($p > 0.05$) were observed in the levels of Tri, HDL, LDL, and VLDL between broiler chicks that were fed local broiler premix and those that were fed commercial diets (Table 3). However, when LBP was replaced at a level of 1.85%, there was a significant ($p < 0.05$) increase in TC levels. The group that had the LBP2 diet had the highest concentration of TC, which was substantially different from the concentration in the CBP1 group. No significant differences were seen in the ratios of TC: HDL, LDL: HDL, and TG: HDL. There is a persistent disagreement among several studies addressing the impact of local broiler premix or used premix sources on lipid profile. These variations could be ascribed to disparities in strain, age, premix amount, and experimental conditions. The findings of this study demonstrated a significant elevation in TC concentrations, although the levels of TG, LDL, VLDL, and HDL remained unaffected by the quantity of local broiler premix supplied to broiler chicks. This increase in serum lipid fractions may be linked to the higher viscosity of the digesta in the intestine and a simultaneous increase in lipid absorption (Capuano, 2017).

Nevertheless, the findings from Zain *al.* (2023) align with the current results, since the inclusion of the premix in broiler diets did not have any impact on the levels of TG, and LDL-cholesterol. Regarding the World Health Organization's recognition of the negative impact of fat accumulation on human health, particularly about life-threatening conditions like atherosclerosis, the favorable lipid profile of chicken meat becomes a significant factor for consumers to consider (Popović *et al.*, 2016).

Table 3. Enzyme activity of structured steroids influenced different levels of experimental diets.

Variables	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	TC:HDL (%)	LDL:HDL (%)	TG:HDL (%)
CBP1	151.50 ^b	84.00	100.50	33.65	16.80	1.52	0.30	0.85
LBP2	182.00 ^a	67.25	118.00	49.75	13.45	1.53	0.43	0.57
LBP3	166.50 ^{ab}	74.00	107.50	47.40	14.80	1.60	0.45	0.67
LBP4	150.75 ^b	71.00	101.00	65.80	14.20	1.50	0.63	0.70
SEM	4.396	5.901	3.070	8.322	1.180	0.040	0.074	0.063
P-value	0.0157	0.811	0.144	0.643	0.811	0.866	0.535	0.542

TC: total cholesterol, TG: total triglyceride, HDL: high-density lipoproteins, LDL: low-density lipoproteins, VLDL: very low-density lipoproteins. CBP1 (basal diet + 2.5% of commercial premix). LBP2 (basal diet + 1.85% of local premix). LBP3 (basal diet + 2.25% of local premix). LBP4 (basal diet + 2.50% of local premix). ^{a,b} Means in the same column with different superscripts are significantly different.

The results, as shown in Table 4, reveal a correlation between peptide and steroid enzymes in chicks that were provided with various types of premix supplementation. A strong positive correlation ($p < 0.01$) was seen between Glu and Alb, TG and VLDL, TG and ALT, VLDL and ALT, as well as TP and ALB. In addition, there was a statistically significant negative correlation ($p < 0.01$) observed between Glu levels and both TG and VLDL levels. Furthermore, there was a considerable and statistically significant positive correlation ($p < 0.05$) observed between Glu and TP, as well as TC and TP.

Table 4. Person correlation of biochemical parameters of broiler chicks.

	Glu	TC	TG	HDL	LDL	VLDL	AST	ALT	TP	ALB
TC	0.21									
TG	-0.63	0.15								
HDL	-0.01	0.51	-0.06							
LDL	0.04	0.28	0.10	0.38						
VLDL	-0.63	0.15	1.00	-0.06	0.10					
AST	-0.23	0.08	0.38	0.32	0.11	0.38				
ALT	-0.18	0.31	0.58	0.29	0.01	0.58	0.10			
TP	0.52	0.51	-0.13	-0.01	0.21	-0.13	-0.10	-0.15		
ALB	0.77	0.24	-0.44	-0.09	-0.09	-0.44	-0.33	-0.25	0.64	
Glo	-0.32	0.30	0.38	0.09	0.35	0.38	0.28	0.12	0.38	-0.45

Glu: glucose, AST aspartate aminotransferase, ALT alanine transaminase, TP: total protein, ALB: albumin, and Glo: globulin, TC: total cholesterol, TG: total triglyceride, HDL: high-density lipoproteins, LDL: low-density lipoproteins, VLDL: very low-density lipoproteins.

The observed correlation between liver enzymes and lipid enzymes might be explained in this way the birds that were given different amounts of local broiler premix showed a positive relationship between serum ALT activities and TG and VLDL levels compared to the control group. This indicates significant liver damage (hepatotoxicity), which was confirmed by the presence of hyperplastic and necrotic biliary epithelium, as well as hepatic degeneration and necrosis in the histopathological analysis (Hashem *et al.*, 2021). The association between blood ALB and TP concentrations is a reliable predictor of hepatic

function. Additionally, when combined with blood AST and ALT levels and blood lipid profile, these indicators are strongly associated with the severity of hepatic lipidosis (Mousa *et al.*, 2023). These findings validate a negative correlation between Glu levels and both TG and VLDL levels. The results presented here are consistent with those of Lv *et al.* (Ly *et al.*, 2022), who likewise observed an adverse correlation between blood TG levels and body fat deposition in meat ducks. The use of a powder diet reduced body fat formation but increased serum TG levels. These results appear to align with a prior study that discovered The levels of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) in broilers have a direct correlation with the accumulation of fat in the carcass (Ghasemi *et al.*, 2016; Barrak *et al.*, 2021).

CONCLUSION

Based on the observed data, it can be concluded that substituting commercial premix with local broiler premix does not have any negative impact on the serum biochemical parameters of broilers. Our study results indicate that local broiler premix can enhance digestion and absorption by supplying crucial amino acids like lysine and threonine. Additional research is required to investigate the optimal parameters that can provide a comprehensive understanding of the utilization of local broiler premixes in chicken diets.

CONFLICT OF INTEREST

Regarding this manuscript, the authors disclose no conflicts of interest.

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