

EFFECTS OF CALCIUM, AVAILABLE PHOSPHORUS AND SODIUM DI-FORMATE LEVELS: 1- PERFORMANCE AND TIBIA MEASUREMENTS OF BROILER CHICKS

S.A. Ibrahim, H.A. El Alaily; M.A.M. Abdelaziz; A.Y.M. Abdelhady and A.I. El-Faham

Poultry Production Department, Faculty of Agriculture, Ain Shams University, Egypt

SUMMARY

An experiment was conducted to determine the effect of adding different sodium di-format (NDF) levels in the presence of low levels calcium and available phosphorus diets on broiler productive performance and tibia measurements. A total number of 140 one day old Hubbard broiler chicks were classified into 7 equal groups, each was subdivided into 4 replicates with 5 chicks. The control group was fed standard (starter and grower) diets formulated ensuring sufficient supply of nutrients suggested by the guidebook of Hubbard broilers that contain 100% (Ca and AP) requirements without (NDF) and a 3 x 2 factorial arrangement with 3 different levels of Ca & AP being (50%, 40% and 30%) from requirements and 2 levels of dietary NDF (1.5 and 2.25 kg/ ton). Feed and water were supplied ad-libitum throughout the whole experiment which lasted till 35 days of age. The results indicated that: Under the condition of the present study, broiler chicks fed control diets or diets containing 50% Ca & AP with 2.25 kg/ton (T4) improved productive performance (live body weight, body weight gain, feed consumption and feed conversion ratio) comparable or better than other treatments. Similar trends were observed regarding the effect of previous dietary treatments on tibia bone measurements (Tibia; length and width) and tibia chemical composition (tibia Ca & P) percentages. However, broiler chicks fed (T4) diets during experimental period (35 days) reflected the lowest significant tibia breaking strength and ash% compared with those fed control diets. It is obvious that, the best performance was seen when 50% Ca & AP 2.25 kg/ton (NDF) were applied in broiler diets without any adverse effect on productive performance and most of tibia measurements and chemical composition.

Keywords: *Calcium, Phosphorus, Sodium diformate, Productive performance and tibia measurements.*

INTRODUCTION

Calcium and phosphorous are couple of essential inorganic materials which are involved in many physiological functions of the body of living poultry (Underwood and Suttle, 2001).

The Ca/AP ratio of 2.2 was found to be optimal for P efficiency (Van der Klis and Gerritsen, 1994), whereas the Ca/ AP ratio should range between 1.5 and 2.2 (Dieckmann, 2004). Moreover, The P utilization is affected by many factors, such as the amount of P in the feed (Günther et al., 1978), the age of the animal and sources of P supply (Günther and Al-Masri, 1988), the amounts of P, Ca and the Ca: AP ratio in the feed (Hermes, 1977) and phytase activity (Schoner et al., 1993) in addition to organic acids supply. It was hypothesized that a high Ca:AP ratio combined with a marginal AP level would be harmful for bone development of broilers. However, low Ca/AP ratio might obstruct bone formation because of a Ca deficiency. However, Deficiencies of calcium and phosphorus lead to their reduction in bone (Wilson and Duff, 1991; Hemme et al., 2005).

Summers (1997) declared that dietary P levels could be reduced by up to 20% for most classes of poultry without any adverse effect on bird's performance. Low levels of Ca and NPP can be fed up to the finisher phase without retarding performance (Dhandu and Angel, 2003). Concerning water consumption, birds fed deficient Ca increase feed and water intake in comparison to those getting sufficient dietary Ca (Damron and Flunker, 1995). In addition, many experiments have reported that dietary organic acids and their salts can increase phosphorus utilization in corn-soybean meal diets fed to broiler chickens (Esmailipour et al., 2011).

The P utilization may be increased due to the chelating properties of organic acids with calcium, which can result in increased phytate-phosphorus solubility, increasing their ability to be hydrolyzed (Centeno et al., 2007).

A new molecule (sodium diformate, similar to potassium diformate) has been proven to be effective against pathogenic bacteria, including salmonella, along the whole gastro-intestinal tract (Hebeler et al., 2000). The reduced impact of pathogenic bacteria on the broiler, as well as the improved gut microflora, leading to a state of eubiosis in treated chickens, suggests that including diformate in broiler diets will also result in improved bird performance. Several trials have been also being carried out over the last half-decade world-wide that document positive effects on broiler performance.

The study by (García et al., 2007; Lückstädt and Wegletner, 2012; Abdelhady et al., 2015) analyzed the average impact from all studies on the effect of the additive sodium diformate on the performance parameters weight gain, feed efficiency, mortality and productive productivity. The final data-set contained the results of 8 documented studies, comprising 17 trials with sodium diformate (NDF) inclusion, which ranged from 0.1% to 0.6%. Results are expressed as percentage difference from the negative control. The average level of dietary NDF from the data-set in all treated broilers was 0.28%. Only a numerical increase of feed intake (1.1%) could be monitored ($P=0.22$). However, the performance of broilers based on daily gain was significantly increased by 5.2% ($P<0.001$). Furthermore, the FCR was also significantly improved (4.1%; $P<0.01$). Survival was increased on average by 2.3% ($P<0.05$). Finally, the European Broiler Index (EBI) improved significantly due to the inclusion of NDF by 12.4% ($P<0.001$). It is therefore concluded that dietary sodium diformate can improve broiler production world-wide (Lückstädt et al., 2014). Moreover, Boling et al. (2000a) reported that increasing level of citric acid up to 6% linearly increased percent tibia ash from 26.9% (P-deficient basal diet) to 38.6%. Suggested that citric acid chelated Ca in the diet and prevent the formation of Ca phytate complexes and therefore increased phytate P utilization with a difference in the response of broilers vs. laying hens to citric acid.

Accordingly, the current study aimed to investigate effects of three dietary Ca and NPP deficiency levels with constant Ca: AP ratios, with two levels of NDF addition as a feed additive in broiler diets, on productive performance and tibia bone characteristic.

MATERIALS AND METHODS

The current study was carried out at the Poultry Nutrition Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University, Qalyubia, Egypt, in order to investigate the role of adaptive non-phytate phosphorus deficiency with sodium diformate (NDF) supplementation on performance and tibia measurements of broilers. A total number of 140 one day old Hubbard broiler chicks were divided into 7 equal treatments groups, each included 4 replicates with 5 chicks each. Experimental treatments consisted of control group plus 3 x 2 factorial arrangement with 3 different levels of Ca x AP being (50%, 40% and 30%) from requirements and 2 levels of dietary sodium diformate (NDF) 1.5 and 2.25 kg/ton, respectively. As presented in Table (1) control diets were formulated ensuring sufficient supply of nutrients suggested by the guidebook of Hubbard broiler that contain 100% Ca & AP requirements without (NDF). Chicks were reared in battery cages and, were fed starter diets from 1 to 14 days of age while, grower diets were fed from 15 to 35 days of age.

Chicks of all experiments were kept under similar hygienic and vaccinated against common diseases. The performance parameters included body weight and feed consumption which were determined weekly and taken body weight gain (BWG), feed conversion ratio (FCR) and performance index were calculated slaughtered then the six tibiae were removed, numbered and frozen at -20° , fat and muscle tissues along with cartilage caps were removed prior to storage. Tibia weight (g), was determined to by using a three decimal digital scale, tibia length and width were determined using a digital micrometer according to the methods described by Samejina (1990).

Tibia breaking strength (TBS) was determined using the method prescribed by Fleming et al. (1998). Proximate chemical analysis of bone was determined by using standard (AOAC, 1995).

Statistical procedures: The collected data were subjected to two way analysis of variance to detected the effects of Ca & AP levels (L) and NDF levels (E) and their interactions (L * E) using the general liner

model (GLM) procedure of SAS (SAS 2002) according to the following model :

T_{ijk} = trait measured, M = Overall mean, L_i = Ca & AP levels, i = (1, 2, 3)

E_j = NDF levels, j = (1, 2), (L * J) ij = Interaction between Ca & AP levels and NDF levels.

E_{ijk} = Experimental error

When significant differences among means were found, means were separated using Duncan's multiple range tests (Duncan, 1955).

Table (1): Feed ingredients and chemical composition of diets (starter period).

Ingredient	Control	T1	T2	T3	T4	T5	T6
Yellow Corn	52.76	55.390	55.81	56.15	55.315	55.735	56.075
Soy bean meal 44 %	30.80	31.75	31.75	32.50	31.75	31.75	32.50
Corn Gluten 60%	9.00	8.00	8.00	7.50	8.00	8.00	7.50
Vegetable Oil	2.57	2.00	2.00	1.85	2.00	2.00	1.85
Ca Carbonate	1.80	0.85	0.66	0.48	0.85	0.66	0.48
MCaP*	1.82	0.63	0.40	0.15	0.63	0.40	0.15
HCl Lysine	0.34	0.32	0.32	0.30	0.32	0.32	0.30
MHA-METH**	0.21	0.21	0.21	0.22	0.21	0.21	0.22
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Anti-mycotoxins***	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium diformate (NDF)	0.00	0.15	0.15	0.15	0.225	0.225	0.225
Premix****	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical Composition (Calculated) #							
CP%	23.01	23.02	23.02	23.03	23.05	23.01	23.00
ME Kcal/Kg	3004	3009	3005	3011	3008	3015	3010
Ca%	1.00	0.50	0.40	0.30	0.50	0.40	0.30
AP%	0.50	0.25	0.20	0.15	0.25	0.20	0.15
Grower diet (15-35 days)							
Ingredients	Control	T1	T2	T3	T4	T5	T6
Yellow Corn	55.08	57.37	57.76	58.91	57.295	57.685	58.835
Soy bean meal 44%	30.00	31.00	31.00	30.70	31.00	31.00	30.70
Corn Gluten 60%	6.00	5.00	5.00	5.00	5.00	5.00	5.00
Vegetable Oil	4.50	4.00	4.00	3.50	4.00	4.00	3.50
Ca Carbonate	1.60	0.77	0.59	0.42	0.77	0.59	0.42
Mono CaP*	1.62	0.52	0.32	0.13	0.52	0.32	0.13
HCl Lysine	0.24	0.22	0.21	0.22	0.22	0.21	0.22
MHA -METH	0.26	0.27	0.27	0.27	0.27	0.27	0.27
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Anti-mycotoxins***	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Formi NDF	0.00	0.15	0.15	0.15	0.225	0.225	0.225
Premix****	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical Composition (Calculated) #							
CP%	21.04	21.06	21.05	21.08	21.03	21.02	21.07
ME Kcal/Kg	3111	3109	3125	3118	3105	3110	3105
Ca%	0.90	0.45	0.36	0.27	0.45	0.36	0.27
AP%	0.45	0.225	0.18	0.14	0.225	0.18	0.14

*MCaP: Mono-Ca-Phosphate; **MHA: Methionine Hydroxyl Analogu; *** Anti-mycotoxins: BASF Novasil™ Plus

**** Each 3 kg of premix containing: 15000000 I.U VIT. A, 50 g. 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 3kg.

RESULTS AND DISCUSSION

Production performance

Productive performance traits of broilers from one to 35 days of age in response to dietary different levels of Ca & AP and NDF are presented in Table (2). The obtained data showed that there were significant differences in LBW and BWG values among treatments during whole experimental period (1-35days) responses of chicks fed diets containing different levels of (Ca & AP) showed that chicks fed diets containing high levels of (Ca & AP), 100% and 5%, supported the highest LBW and BWG compared with those fed the two other levels (40% and 30%). The corresponding figures were 1628 and 1569 g versus 1489 and 1416 g respectively for LBW, 1586 and 1527 g versus 1447 and 1376 g respectively for BWG with significant differences between treatments. Levels of NDF showed the same trend since the higher LBW and BWG were detected for the chicks fed 2.25 kg/ton NDF diets compared with chicks fed 1.50 kg/ton NDF diets and the corresponding figures were 1582 and 1540 g versus 1395 and 1355 g respectively with significant differences between the two treatments. In addition, feeding (T4) diets showed the highest LBW (1634 g) and BWG (1592) followed by those fed control diets being 1628 g and 1586 g, respectively. Besides, the differences between the two treatments were insignificant.

These results might be justified by several reasons; First: Formic NDF might inhibit intestinal bacteria that competing with the host for available nutrients, and a reduction of possibly toxic bacterial metabolites, thus improving LBW and BWG of chicks. Second, lower ileum pH might increase nutrient availability.

Data of broiler body weight, body weight gain, from both experiments were commonly, in agreement with the results of (Bozkurt et al., 2009). Other authors reported that formic acid (0.1 and 1.0%) had positive effects on growth performance of broilers (Helen and Christian, 2010). While, Hernandez et al. (2006) reported that formic acid (0.5 to 1 kg/ ton) did not affect LBW and BWG of broilers that may be due to low levels of acids non-sufficient to cause performance improvement.

Feed consumption (FC) and feed conversion ratio (FCR)

Data presented in Table (2) indicated that FC per chick (gram/chick) was significantly decreased by feeding low Ca & AP level (30%) compared with those fed other treatments (100, 50 or 40%).

The corresponding figures were 231 g versus 2570, 2536 and 2429 g with significant differences between

Table (2). Effect of different dietary treatments on growth performance of broiler chicks.

	Treatment	LBW		BWG	FC	FCR	PI
		1 day	35 days				
Ca&AP levels %	100	41.75	1628a	1586a	2570 ^a	1.62	100.49a
	50	41.40	1565b	1523a	2506a	1.65	94.85a
	40	41.48	1484c	1442b	2406ab	1.67	88.86b
	30	39.74	1419c	1379c	2305b	1.67	84.97b
NDF levels	0.00	41.75	1628a	1586a	2570a	1.62b	100.49
	1.50	39.97	1395b	1355b	2213b	1.63b	85.58
	2.25	41.77	1583a	1541a	2598a	1.69a	93.67
Interaction	CONTROL	41.75	1628a	1586a	2570a	1.62	100.49a
	T1	40.65	1495c	1454b	2385b	1.64	91.16ab
	T2	39.6	1393d	1353c	2179c	1.61	86.52b
	T3	39.65	1297e	1257d	2075d	1.65	78.61b
	T4	42.15	1634a	1592a	2627a	1.65	99.03a
	T5	43.35	1574ab	1531ab	2633ab	1.72	91.51a
	T6	39.82	1540b	1500ab	2535a	1.69	91.12ab
SIG.	Ca & AP	NS	**	**	*	NS	*
	NDF	NS	**	**	**	*	NS
	INTERACTION	NS	**	*	*	NS	*

a' b c, d Means within the same column with different superscripts are significantly different.

*Sig. = Significance ** (P<0.01), * (P<0.05). NS = Non-Significant.*

LBW = live body weight, BWG= Body weight gain, FC= feed consumption, FCR= feed conversion ratio

treatments. In the same order during whole experimental period, chicks fed diet containing low level of NDF (1.50 kg/ton) significantly decreased FC compared with that fed high level of NDF (2.25 kg/ton), the corresponding figures were 2213 versus 2598 g with significant differences between treatments. In addition, FC per chick decreased by feed T1-3 diets compared with those fed control or T4-6 diets, with significant differences feed conversion ratio (FCR), the figures of FCR indicated insignificant differences between chicks fed different levels of Ca & AP.

The best FCR was detected for the chicks fed control diets (100%) being (1.62) compared to other dietary treatments 50, 40 or 30% (being, 1.66, 1.67 and 1.68 respectively), however, the differences failed to be significant. In the same order, the worst FCR was detected for the chicks fed diets incorporated with (1.50 kg/ton) NDF compared with chicks fed higher level of NDF (2.25 kg/ton), the corresponding figures were 1.68 versus 1.63 respectively. Besides the differences between the two treatments were significant. Moreover, the figures of FCR indicated insignificant differences between chicks fed diets containing low levels of Ca & AP with different levels of NDF (T1-6) compared with those fed control diet. The least FCR was detected for the chicks fed T2 (1.61) or control (1.62) diets. On the other hand, the worst FCR were found in chicks fed T6 (1.69) or T5 (1.71) diets and differences between treatments failed to be significant (Table 2).

Increased feed intake in (control), (T4), (T5) and (T6) groups could be explained by three reasons. First, broilers fed diet with low Ca and P showed maximized FC and poorer FCR in a try to manage LBW according to Waldroup et al. (2000) and Rouhollah et al., (2010).

Second, birds might adapt to sufficient Ca and P by raising FC that caused worse FCR as Kheiri and Rahmani (2006) who suggested that using low levels of both Ca and P up to 30% of requirement suggested levels, would affect FCR negatively. Third reason, Formic NDF supplementation might increase FC that raised FCR. This was in accordance with Adil et al. (2011) who stated that addition of 2% formic acid, showed significantly ($p < 0.05$) higher body weight gains and feed conversion ratio Hernandez et al. (2006); Helen and Christian (2010).

Tibia measurements:

The results in Table (3) show the relationship between dietary treatments and some tibia measurements and tibia breaking strength (TBS). The percentages of tibia weight in relation to LBW for broiler chickens fed control diets reflected insignificant differences than those fed different dietary treatments (T1-6). On the same order, tibia length (cm) and tibia width (mm) were almost the same when chickens fed control or T1-6 diets. The response to feeding low levels of Ca & AP with different levels of NDF on tibia measurement showed insignificant differences without clear trend in the relation to the levels of Ca & AP or levels of NDF.

In general, the figures of tibia measurement were seen when 30% Ca & AP with 2.25 kg NDF/ton (T6) was incorporated in diets the corresponding values were 1.00%, 10.43 cm and 2.53 mm for tibia weight %, tibia length and tibia width respectively. Chemical composition of tibia bone for broiler chick as affected by experimental treatments is illustrated in Table (3). The obtained data showed that there were significant differences in Tibia (Ash, Ca and P) percentages among treatments. Birds fed control diets reflected the highest figures compared with other treatments (T1-6).

However, Ash % decreased by (22.47%, 22.31% and 31.13%) for chicks fed (50%, 40% and 30%) Ca & AP levels diets compared with those feed control diets and Tibia (Ca and P) percentages showed similar trend. Besides, the differences between treatments were significant. Moreover, feeding diets containing 2.25 kg/ton (NDF) gave the highest Ash % (37.03 vs. 34.96), Ca% (13.52 vs. 13.23) and P% (8.96 vs. 8.32) compared the diets containing 1.52 kg/ton (NDF), however, the differences failed to be significant except ash %. In the same order, dietary NDF levels with different Ca & AP levels showed that control, T1, T2, T4 and T5 groups appeared significantly ($P > 0.05$) similar and higher than other treatments T3 and T6, except ash%. On the other hand, data showed that chicks fed control diets had highest values and chicks fed (T3) had the lowest values of ash, Ca and P percentages. Besides, the differences between the two treatments were significant. In accordance with Yan et al. (2001) who indicated that birds fed control diet had higher ash weight of P or Ca consumed at all ages as compared with that of birds fed the deficient Ca and P treatments, this could be attributed to considerable adaptation to P and Ca restriction in deficient Ca and P treatments. And bond mineral content of birds at control treatments had higher values. Additionally, Rafacz-Livingston et al. (2005) employed that citric acid to improve P utilization as well as tibia Ca & P content and

enhanced bone strength in broilers by improving total digestibility of Ca and P (Snow et al., 2004 and Islam, 2012).

Table (3) Effect of different dietary treatments on tibia measurements of broiler chicks.

	Treatment	Weight %	Length (cm)	Width (mm)	TBS ## (Kg/cm ²)	Ash %	Ca %	P %
Ca & AP levels	100	1.02a	9.18a	7.95	43.55a	14.57a	14.57a	9.49a
	50	0.93ab	8.77b	7.65	34.69b	13.76ab	13.76ab	8.90b
	40	0.93ab	8.73b	7.44	34.19b	13.66b	13.66b	8.68b
	30	0.86b	8.17b	7.94	32.91b	12.72c	12.72c	8.35b
NDF levels	0.00	1.02a	9.18a	7.95	43.55a	14.57a	14.57a	9.49a
	1.50	0.89b	8.48b	7.84	33.72b	13.23b	13.23b	8.32b
	2.25	0.92b	8.63b	7.51	34.14b	13.52b	13.52b	8.96b
INTERACTION	CONTROL	1.02a	9.18	7.95	43.55a	14.57a	14.57a	9.49a
	T1	0.89b	8.29	7.54	34.47b	13.41ab	13.41ab	8.76a
	T2	0.96a	9.01	7.66	33.90b	13.87ab	13.87ab	8.66ab
	T3	0.81b	8.13	8.31	32.78b	12.42b	12.42b	7.54b
	T4	0.97a	9.24	7.75	34.91b	14.11ab	14.11ab	9.03a
	T5	0.90ab	8.44	7.22	34.48b	13.44ab	13.44ab	8.69a
	T6	0.90ab	8.20	7.56	33.03b	13.02b	13.02b	9.15a
SIG.	Ca & AP	*	*	NS	*	**	**	*
	NDF	*	*	NS	*	**	*	*
	INTERACTION	*	NS	NS	*	*	*	*

a' b c Means within the same column with different superscripts are significantly different.

*Sig. = Significance ** (P<0.01), * (P<0.05). NS = Non-Significant. - TBS= Tibia breaking strength*

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تأثير المستويات المختلفة للكالسيوم والفسفور المتاح وأملاح فورمات الصوديوم على 1- الأداء الإنتاجي وقياسات عظمة الساق لدجاج اللحم

سيد عبدالرحمن ابراهيم - حسين عبدالله العلايلي - مروان عبدالعزيز محمود عبدالعزيز- عبدالرحمن يوسف محمد عبدالهادي- أحمد ابراهيم سليمان الفحام

قسم إنتاج الدواجن- كلية الزراعة- جامعة عين شمس- القاهرة- مصر

أجريت تجربة للتعرف على تأثير إضافة مستويات مختلفة في أملاح فورمات الصوديوم إلى علائق منخفضة المحتوى من الكالسيوم والفسفور المتاح على الأداء الإنتاجي وقياسات عظمة الساق لدجاج اللحم. استخدم في التجربة 140 كتكوت هبرد عمر يوم وزعت على 7 معاملات غذائية (4 مكررات/ 5 كتكوت بكل مكرر) وكانت المعاملات كالاتي: كتنترول (عليقة قياسية) تغطي 100 % للاحتياجات من الكالسيوم و الفسفور المتاح بدون إضافة فورمات الصوديوم و6 معاملات غذائية 3 × 2 (3 مستويات كالسيوم و فوسفور متاح بنسبة 50 و40 و30% من العليقة القياسية) و2 مستوى من أملاح فورمات الصوديوم (1.5 و 2.25 كيلو جرام/ طن). وتم توفير العلف والماء بصورة حرة للطيور طول الفترة التجريبية (35 يوم). و كما أنهم النتائج:

- سجلت الطيور المغذاة على عليقة الكتنترول أو عليقة T4 (50% في الاحتياجات كالسيوم & فوسفور المتاح + 2.25 كيلو فورمات صوديوم / طن) أفضل نتائج للأداء الإنتاجي (وزن الجسم والوزن المكتسب واستهلاك العلفومعامل تحويل غذائي) بالمقارنة بالمعاملات الأخرى.

- سجلت الطيور المغذاه على عليقة الكتنترول أو عليقة T4 أفضل نتائج لقياسات عظمة الساق (طول وسمك عظمة الساق) والتركيب الكيميائي (عظمة الساق (كالسيوم والفسفور%) إلا أن الطيور المغذاه على عليقة (T4) سجلت أقل قيم معنوية لقوة الكي والمحتوى في الرماد % لعظمة الساق بالمقارنة بمجموعة المغذاه على عليقة الكتنترول. لذلك فإن إضافة 2.25 كيلو فورمات الصوديوم/ طن علف دجاج اللحم المنخفض في المحتور في الكالسيوم والفسفور المتاح (50%) عن الاحتياجات يحسن الأداء الإنتاجي ومعظم قياسات عظمة الساق.