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Effects of Dietary Calcium, Phosphorus, Biotin, and Fat on the Performance and Nutrient Utilization of Meat Chickens

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Abstract. The effects of different dietary levels of calcium (Ca), available phosphorus (AP), biotin, fat and different types of fat on 5-to 19-day growth performance, liver biotin concentration, and nutrient utilization of meat chickens were investigated in two experiments. A high Ca diet (25.4 vs. 12.2 g/kg) reduced growth and feed efficiency of meat chickens, while a high Ca and AP diet (25.7 & 11.9 g vs. 12.2 & 5:2 g) caused further depression in performance. Neither 125 ug biotin/kg diet nor 4% tallow, 4% vegetable oil or a mixture of 2% vegetable oil and 2% tallow appeared to completely overcome the growth depression caused by a high Ca diet. The addition of 125 ug/kg biotin in combination with 4% vegetable oil or a mixture of 2% vegetable oil and 2% tallow, appeared to completely overcome the growth depressing effect of a high Ca diet but not of a high Ca and high phosphorus diet. It was concluded that the addition of biotin and vegetable oil to high Ca diets was successful in preventing growth depression.

Introduction

There are several dietary constituents which can influence calcium (Ca) availability and, consequently, requirement and maximum tolerated levels of Ca [1]. Phosphorus (P) and fat can reduce Ca availability and possibly reduce the adverse effects of high Ca diets [2-6]. The effects of varying the dietary Ca and available phosphorus (AP) on chickens performance and Ca metabolism has been the topic of continuing research [1, 2]. Ca reacts with fat in the digestive tract resulting in the formation of Ca soaps which are excreted [5, 7]. The formation of insoluble soaps from divalent cations and fatty acids is an important aspect of poultry nutrition because it influences both fatty acid metabolism and the availability of Ca. The relationship between dietary levels of Ca and biotin was investigated by Bryden and Balnave [8] who showed that the addition of 75 ug of biotin/kg diet improved weight gain, feed intake and feed efficiency of growing chickens fed high Ca diets. This level of biotin is about 50% of the standard requirement [9]. Two experiments were undertaken to investigate the effects of fat and biotin on the performance of chickens fed diets containing different Ca and AP levels.

Materials and Methods

Experiment 1: This experiment was designed to investigate the effects of fat and biotin supplementation of diets containing high levels of Ca and AP on bird performance and liver biotin concentration. The experiment was a 3 x 2 x 2 factorial, with factors of Ca and AP levels (12.2 and 5.2g, 25.4 and 5.2g or 25.7 and 11.9 g/kg diet, respectively), added fat (0 or 4% sunflower oil) and added biotin (0 or 125 ug/kg diet). A total of ninty-six, unsexed commercial meat chickens were fed a commercial diet until five days old. At five days of age, the chickens were weighed and individually caged with a separate feeding trough in an electrically heated battery brooder. Each experimental diet was fed to eight chickens. The composition of the basal diets without and with oil are shown in (Table 1). Dietary energy and protein content were kept approximately constant by substituting for wheat in the first basal diet, wheat bran and sunflower oil in the second basal diet. High Ca or high Ca and AP levels were achieved by the substitution of limestone and dicalcium phosphate for an equal amount of wheat. Food and water were supplied ad libitum. The birds were weighed again at sixteen days of age when the experiment concluded. Feed intake was measured over the eleven day experimental period. At the end of the experiment, two chickens per treatment were killed by cervical dislocation and livers were removed and immediately frozen at -15°C prior to analysis. Liver biotin concentration was determined by the method of Hood [10]. Measurements were made of individual body weight gain, feed consumption, feed conversion ratio (FCR) and liver biotin concentration.

Experiment 2: This was designed to investigate the effects of type of fat and biotin supplementation on nutrient utilization of chickens fed diets containing different Ca and AP levels. Excess dietary concentrations of Ca and AP were formulated in this experiment to be less than those used in the previous experiment. The experiment was a 3 x 4 x 2 factorial, with factors of: dietary Ca and AP levels (15.3 and 4.3g, 21.8 and 4.3g or 22.6 and 8.3 g/kg diet, respectively), added fat (0, 4% beef tallow, 4% vegetable oil or a mixture of 2% beef tallow and 2% vegetable oil) and added biotin (0 or 125 ug/kg diet). A total of 192 unsexed commercial meat chickens were fed a commercial diet until eight days old. At eight days of age, the chickens were weighed and housed under similar conditions to those described in experiment 1. Each experimental diet was fed to eight chickens. The compositions of the basal diets are shown in (Table 1). Dietary energy and protein contents were kept approximately constant by substituting parts of the major feed ingredients with wheat bran and oil or tallow. High Ca or high Ca and AP levels were achieved by the substitution of limestone and dicalcium phosphate for an equal amount of wheat.

Table 1. The composition of the basal diets (g/kg)

	Experiment					
	1		2			
Ingredient						
Wheat	791.45	641.45	442.3	442.3	362.3	402.3
Sorghum	-	-	290.0	210.0	180.0	180.0
Cottonseed meal	-	-	50.0	40.0	40.0	40.0
Soy bean meal	100.00	100.00	80.0	80.0	60.0	60.0
Bran	-	110.00	-	50.0	190.0	150.0
Meat meal (53% protein)	40.00	40.00	50.00	50.0	50.0	50.0
Fish meal (65% protein)	50.00	50.00	40.00	40.0	30.0	30.0
Skim milk powder	-	-	30.00	30.0	30.0	30.0
Tallow	-	-	-	40.0	-	20.0
Sunflower oil	-	40.00	-	-	40.0	20.0
Limestone	2.05	2.05	10.00	10.0	10.0	10.0
Dicalcium phosphate	10.00	10.00	-	-	-	-
Sodium chloride	1.50	1.50	1.2	1.2	1.2	1.2
Celite (insoluble ash)	-	-	2.0	2.0	2.0	2.0
Premix1	5.00	5.00	4.5	4.5	4.5	4.5
Analysis						
Crude protein (N% x 6.25)	20.3	19.9	21.3	20.7	20.4	20.2
Fat %	2.94	7.01	3.2	7.0	8.0	7.9
Calcium %	1.22	1.24	1.53	1.52	1.43	1.47
Total phosphorus %	0.68	0.72	0.76	0.75	0.72	0.74
Calculated available phosphorus[2] %	0.50	0.51	0.43	0.44	0.45	0.44
Calculated metabolizable energy[3] (kj/g)	12.4	12.7	12.1	12.5	12.2	12.2

[1] The composition of vitamins and minerals in the premix (per tonne of diet): Retinol, 12g; cholecalciferol, 4g; D-L tocopheryl acetate, 10g; riboflavin, 3g; biotin, 0.5g; pyridoxine HCl,0.5 g; menadione sodium bisulphite, 1.0g; zinc bacitracin (10%), 2.0 g; manganese sulphate, 60 g; zinc oxide, 50 g; ethoxyquin (33%), 150 g; DL-methionine, 500 g; L-lysine hydrochloride, 1 000 g; furazolidone, 100 g; carrier (pollard), to 5 kg.

[2] Available phosphorus was calculated on the basis of 30% availability of phosphorus in plant products.

[3] Metabolizable energy was calculated from Evans [11].

The birds were weighed again at 19 days of age when the experiment concluded. Feed intake was measured over the eleven day experimental period. Excreta was collected every 12 hours in the last two days of the experiment and acidified by 0.5 M sulphuric acid (to prevent any microbial action causing loss of ammonia). Feed samples and excreta were oven dried at 100°C and finely ground prior to analysis. Nitrogen in both feed and excreta was determined by the Kjeldahl procedure [12], gross energy was determined by using an adiabatic bomb calorimeter, dietary acid-insoluble ash was used as a marker and determined by the method of Vogtman et al. [13] and the faecal nitrogen was separated by the uranyle acetate precipitation method of Ekman et al. [14]. Fat was determined by extraction for 18 hours with 60-80°C petroleum spirit using the soxhlet apparatus. Faecal soap was determined using the principle of fat determination in faeces reported by Carroll [15]. The calculations of nutrient utilization were as described by Scott *et al.* [3]. Measurements were made of body weight gain, feed consumption, FCR, total fat excretion, faecal soap excretion, nitrogen corrected metabolizable energy (ME), digestibility and retention of nitrogen. Data collected was subjected to analysis of variance [16]. Where significant variance ratios were detected, differences between treatment means were tested using the least significant difference (LSD) procedure.

Results

Experiment 1: Results of experiment 1 are presented in (Table 2) and (Figs. 1 and 2).

Table 2. The effects of fat and biotin supplementation on the weight gain, feed consumption and feed conversion ratio and liver biotin concentration of chickens fed diets containing different dietary calcium and available phosphorus levels (Experiment 1)

Diet	Weight gain (g)	Feed intake (g)	FCR[1] (feed/gain)		Liver biotin concentration (ng/g liver)
Ca[2]& AP[3] level (g/kg)					
12.2 & 5.2	231.1^{a}	389.0^{a}	1.61^{c}		901.5^{ab}
25.4 & 5.2	197.5^{b}	367.2^{b}	1.76^{b}		717.3^{b}
25.7 & 11.9	180.7^{c}	353.1^{b}	1.85^{a}		1012.1^{a}
LSD[4](P< 0.05)	14.4	17.6	0.07		292.4
Fat					
Control diet	197.0	363.1	1.75		657.4
Added fat[5]	209.2*	376.5	1.73		1096.5**
Biotin					
Control diet	206.9	367.2	1.71		792.8
Added biotin[6]	199.3	367.5	1.78*		961.1*
SEM[7](84)	4.2	5.1	0.02	(12)	39.2
Interaction					
Ca & AP x Biotin	P < 0.01	-	-		P < 0.07

* Significant difference ($P < 0.05$); ** Significant difference ($P < 0.01$);
1 Feed conversion ratio; 2 Calcium; 3 Available phosphorus;
4 Least significant difference ($P < 0.05$); 5 Added 4% sunflower oil;
6 Added 125ug biotin/kg diet;
7 Standard error of means; degrees of freedom in parenthesis.
a,b,c Within columns, means followed by different superscripts are significantly different ($P < 0.05$).

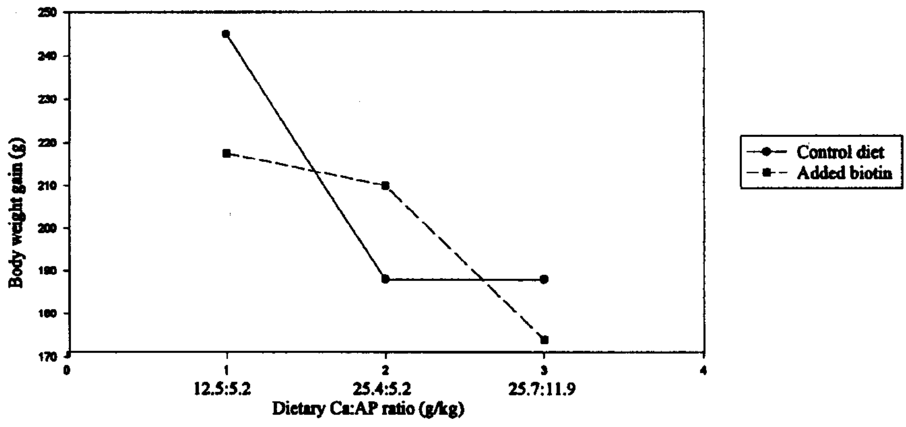

Fig. 1. The effects of biotin supplementation of diets containing different Ca:AP ratios on body weight gain (experiment 1).

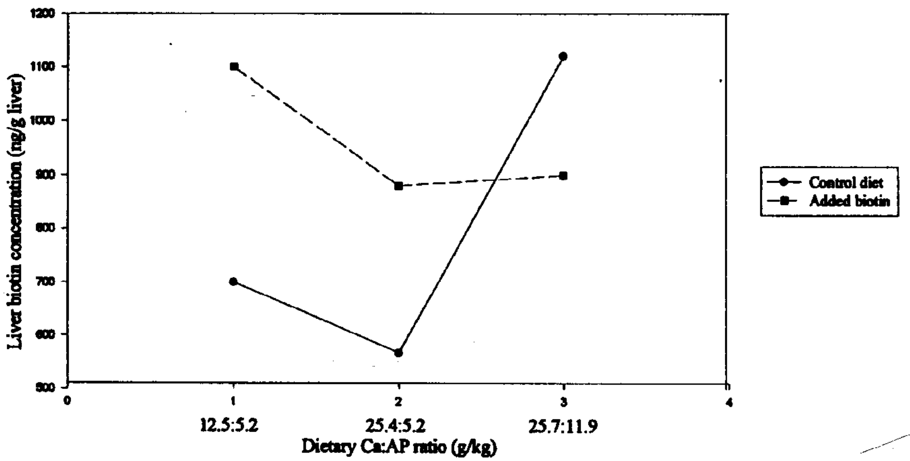

Fig. 2. The effects of biotin supplementation of diets containing different Ca:AP ratios on liver biotin concentration (experiment 1).

The diets with increased Ca significantly reduced body weight gain ($P < 0.01$) and feed consumption ($P < 0.05$) and increased FCR ($P < 0.01$), while increased dietary levels of Ca and P significantly depressed body weight gain and feed consumption and increased FCR ($P < 0.01$). The reduction in body weight gain and the increase in FCR caused by high dietary concentrations of both Ca and P were greater than by high Ca alone ($P < 0.05$). The diet with increased Ca only, produced a lower biotin concentration in the liver than did the diet with increased Ca and P together ($P < 0.05$). The reduction caused by the high Ca diet was not significant when compared with the control diet. Increased dietary fat significantly increased body weight gain ($P < 0.05$) and liver biotin concentration ($P < 0.01$). The addition of biotin to the diet significantly ($P < 0.05$) increased FCR and liver biotin concentration. The addition of biotin to the high Ca diet significantly ($P<0.05$) increased liver biotin concentration and ($0.10 > P > 0.05$) improved body weight gain of chickens, but liver biotin concentration and weight gain of chickens were reduced when biotin was added to the high Ca and high P diet. The interaction between dietary Ca and AP level and biotin for body weight gain showed that the addition of biotin to high Ca diets tended to improve weight gain, but did not completely overcome the depression caused by the excess Ca, while the addition of biotin to the high Ca and high P diet depressed body weight gain further (Fig. 1).

The results from liver biotin measurements indicated that the addition of either biotin or high Ca and P to the diet was positively related to liver biotin concentration, while dietary Ca on its own was negatively related to liver biotin concentration. The interaction between dietary Ca and AP level and biotin for liver biotin concentration showed that the addition of biotin to the high Ca diet increased biotin concentration in the liver, whilst the addition of biotin to the high Ca and P diet reduced it (Fig. 2).

Experiment 2: The effects of type of fat and biotin supplementation of diets containing different Ca and AP levels on the performance of chickens and nutrient utilization are shown in (Tables 3 to 6). The main dietary treatments did not influence chicken weight gain, feed consumption or feed conversion ratio (Table 3). However, there was a significant interaction ($0.10 > P > 0.05$) between dietary concentrations of Ca, AP and dietary biotin and type of fat for body weight gain. The addition of biotin (125 ug/kg diet) to either 4% vegetable oil or a fat mixture (2% tallow and 2% vegetable oil), completely overcame the growth depression caused by a high Ca diet, but not by a high Ca and high P diet (Table 4). Increasing dietary concentrations of Ca or Ca and P significantly reduced nitrogen digestibility ($P < 0.01$) but did not influence nitrogen retention. The addition of a vegetable oil or fat mixture significantly reduced nitrogen digestibility ($P < 0.01$) and nitrogen retention ($P < 0.05$), while tallow supplementation on its own increased nitrogen digestibility ($P < 0.01$). Dietary treatments did not significantly affect metabolizable energy (Table 5). The effects of dietary treatments on fat excretion are presented in (Table 6). Increased dietary Ca or Ca and P significantly increased faecal soap ($P < 0.01$ and $P < 0.05$ respectively), without any effect on total fat excretion. The addition of fat to the diet significantly increased total fat excretion ($P < 0.01$), while type of fat influenced the partitioning of fat excretion between faecal soap and neutral fatty acids. The inclusion of tallow or vegetable oil significantly increased faecal soaps ($P < 0.05$) and neutral fat and fatty acids excretions ($P < 0.01$ and $P < 0.05$ respectively), whereas the mixture of tallow and vegetable oil significantly ($P < 0.01$) increased neutral fat and fatty acids excretion only and did not significantly change faecal soap excretion.

Table 3. The effects of type of fat and biotin supplementation on the weight gain, feed consumption and feed conversion ratio of chickens fed diets containing different calcium and available phosphorus levels (Experiment 2)

Diet	Weight gain (g)	Feed intake (g)	FCR[1] (feed/gain)
Ca[2]& AP[3] levels (g/kg)			
15.3 & 4.3	221.6	437.7	2.04
21.8 & 4.3	212.6	422.6	2.05
22.6 & 8.3	210.0	429.9	2.09
LSD[4] (P <0.05)	19.9	28.5	0.19
Fat			
Control	219.8^{ab}	435.3	2.03
Added 4% tallow	222.3^{a}	431.1	2.01
Added 4% vegetable oil	196.6^{b}	421.4	2.22
Added fat mixture[5]	220.2^{a}	432.4	2.01
LSD[4](P <0.05)	23.3	32.7	0.23
Biotin			
Control diet	214.5	435.2	2.09
Added biotin6	214.9	424.9	2.04
SEM[7](168)	5.9	8.4	0.12
Interaction			
Fat x Biotin	*	-	*

* Significant difference ($P < 0.05$); [1] Feed conversion ratio; [2] Calcium;
[3] Available phosphorus; [4] Least significant difference ($P <0.05$).
[5] Added 2% tallow + 2% vegetable oil; [6] Added 125 ug biotin/kg diet.
[7] Standard error of means; degrees of freedom in parenthesis;
[a,b] Means within column followed by different superscripts are significantly different ($P < 0.05$).

Table 4. The interaction between type of fat and biotin on the weight gain of chickens fed diets containing different calcium and available phosphorus levels (Experiment 2)

Dietary fat	Dietary calcium and available phosphorus (g/kg)					
	15.3 & 4.3		21.8 & 4.3		22.6 & 8.3	
	(-)	(+)	(-)	(+)	(-)	(+)
Control diet	231^{ab}	230^{ab}	214^{ab}	195^{ab}	230^{ab}	215^{ab}
Added 4% tallow	249^{a}	213^{ab}	238^{ab}	203^{ab}	224^{ab}	206^{ab}
Added 4% vegetable oil	178^{b}	199^{ab}	181^{b}	230^{ab}	182^{a}	207^{ab}
Added fat mixture [2]	234^{ab}	235^{ab}	199^{ab}	237^{ab}	206^{ab}	207^{ab}
LSD[1] ($P < 0.05$) = 58.3; LSD[1]($P < 0.1$) = 46.9						

[-] No added biotin; [+] Added 125 ug biotin/kg diet; [1] Least significant difference;
[2] Added 2% tallow + 2% vegetable oil.
[a,b] Means within row followed by different superscripts are significantly different ($P < 0.05$).

Table 5. The effects of type of fat and biotin supplementation of diets containing different calcium and available phosphorus levels on nitrogen digestibility,retention and metabolizable energy (Experiment 2)

Diet	Nitrogen digestibility (%)	Nitrogen retention (mg N/g feed)	Nitrogen corrected metabolizable energy (MJ/kg)
Ca & AP levels (g/kg)			
15.3 & 4.3	90.54^{a}	17.05	12.01
21.8 & 4.3	89.21^{b}	17.75	12.39
22.6 & 8.3	88.69^{b}	17.50	12.10
LSD[1] (P < 0.05)	0.8	1.18	0.57
Fat			
Control	91.86^{b}	18.41^{a}	11.97
Added 4% tallow	96.94^{a}	18.26^{a}	12.47
Added 4% vegetable oil	86.76^{d}	16.58^{b}	12.53
Added fat mixture[2]	88.34^{c}	16.48^{b}	12.23
LSD[1] (P < 0.05)	0.94	1.35	0.66
Biotin			
Control	89.2	17.34	12.10
Added biotin[3]	89.7	17.53	12.20
SEM[4](24)	0.9	0.30	0.19

[1] Least significant difference (P <0.05); [2] Added 2% tallow + 2% vegetable oil;
[3] Added 125 ug biotin/kg diet; [4] Standard error of means; degrees of freedom in parenthesis;
[a,b,c,] Within columns, means followed by different superscripts are significantly different (P < 0.05).

Discussion

Results from both experiments (Tables 2 and 3) are in agreement with Shafey[1] who showed that increasing the dietary Ca level above 22 g/kg with 5 g/kg of AP, reduced body weight gain and increased feed conversion ratio, while increasing AP from between 5 and 10 g/kg tended to reduce the effects of increased dietary Ca on chicken performance. However, increasing dietary AP above 10 g/kg with Ca level of 25 g/kg tended to cause a greater depression in chicken performance than Ca alone. The addition of 125 ug/kg biotin to the diet did not influence weight gain. Whitehead and Bannister [17] concluded that 170 ug biotin /kg diet was necessary for maximum growth. The authors found that with 100 ug/kg biotin, about 97% of maximum weight gain was achieved. Whilst, Guler *et al.* [18] achieved a significant increase in broiler final weight of 4-7% after the addition of 200 ug/kg of biotin to a wheat based diet. The interaction between dietary concentrations of Ca and AP and biotin showed that the effect of dietary biotin supplements on the performance of chickens fed diets containing high levels of Ca is probably determined by the dietary level of AP.

Table 6. The effects of type of fat and biotin supplementation of diets containing different calcium and available phosphorus levels on fat excretion (Experiment 2)

Diet	Neutral fat fatty acids % (F1)	Soap % (F2)	Total (F1+F2)
Ca & AP levels (g/kg)			
15.3 & 4.3	7.65	1.31^{b}	8.92
21.8 & 4.3	6.45	2.37^{a}	8.82
22.6 & 8.3	7.39	2.18^{ab}	9.57
LSD[1](P < 0.05)	1.73	1.03	1.08
Fat			
Control	4.57^{c}	1.04^{c}	5.61^{c}
Added 4% tallow	8.45^{ab}	2.79^{a}	11.24^{a}
Added 4% vegetable oil	6.61^{b}	2.52^{ab}	9.13^{b}
Added fat mixture[2]	9.03^{a}	1.36^{bc}	10.39^{a}
LSD[1](P < 0.05)	2.02	1.19	0.90
Biotin			
Control	7.63	1.69	9.32
Added biotin[3]	6.70	2.16	8.85
SEM[4](24)	0.41	0.24	0.26

[1] Least significant difference (P <0.05); [2] Added 2% tallow + 2% vegetable oil;
[3] Added 125 ug biotin/kg; [4] Standard error of means; degrees of freedom in parenthesis.
[a,b,c] Within columns, means followed by different superscripts are significantly different (P < 0.05).

Biotin has however been reported to overcome the growth depression caused by high Ca diets when the basal diet was deficient in biotin [8]. These authors based their diet on wheat and meat meal. They reported that mortality was high from fatty liver and kidney disease (FLKS) in chickens fed the basal diet. Karunajeewa [19] and Payne [20] showed that biotin responsive disease or deficiency symptoms occurred when birds ingested diets having a substantial amount of meat meal. They showed that biotin deficiency occurred when wheat based diets were fed but not when maize based diets were fed. Excess dietary Ca caused disturbance of kidney function [21]. Karunajeewa [19] suggested that excess of dietary Ca aggravates the symptoms of biotin deficiency. While available biotin content of diets may be calculated, the accuracy of this depends upon limited information on the level and availability of biotin in feedstuffs. Thus while the total level of biotin in reported experiments is not known for certain, both wheat and meat meal are very variable in biotin content and in this trail may have contained much lower levels of biotin than estimated. Although the level of biotin added in this experiment was higher than the level used by Bryden and Balnave [8], (125 ug vs. 75 ug/kg diet), it caused some improvement to the performance of chickens fed high Ca diets. It appears that the effect of adding biotin to the diet was dependent on basal dietary biotin level and on the level of chicken performance on the basal diet. Body weight gain and feed conversion ratio were not influenced by the level or type of fat when diets were maintained isocaloric and isonitrogenous in experiment 2, while the increase in weight gain caused by the addition of fat in the first experiment was probably due to the slight increases in the dietary energy:protein ratios.

The interaction between dietary Ca and AP, biotin and fat found in the second experiment indicated that the addition of biotin to either vegetable oil or a fat mixture completely overcame the growth depression produced by the high Ca diet (Table 4). This supports the conclusion from previous work that wheat and meat meals may either be lower in biotin than calculated or that wheat, meat meal and Ca interfere with biotin utilization. As high Ca decreases fat availability by the formation of insoluble soap, a higher fat level will not prevent soap formation but will enable more fat to be available for absorption. Analysis of nutrient utilization (Table 5) showed that chickens fed increased dietary Ca or Ca and AP at the levels used in this experiment, tended to metabolize more energy per unit of feed compared with chickens fed the control diet. Nitrogen retention followed a similar pattern. Although there was no significant effect on body weight or feed conversion ratio, there was an indication that body weight gain and feed efficiency were reduced with these diets. These results suggest that there is a difference between the efficiency of utilization of metabolizable energy and retained nitrogen due to increased dietary Ca or Ca and AP. Significant decreases in dietary nitrogen digestibility with high Ca diets would suggest that there were differences in the relative absorption by the intestine due to increased dietary Ca or Ca and AP. It appears that chickens may have been able to compensate for the decrease in the efficiency of digestion and absorption of nitrogen and energy by increasing efficiency of their utilization of nitrogen and energy. Data from Table 6 indicates that increasing dietary Ca level increased the formation of insoluble soap. These results are consistent with the findings of others [4,5]. Increasing dietary Ca has also been shown to reduce fat digestibility [22].

It was concluded that both biotin and fat absorption were reduced by high Ca diets, however neither biotin nor type of fat alone appeared to completely overcome the growth depression caused by high Ca diets. Dietary level of AP appeared to influence the effect of biotin on the performance of birds fed a high Ca diet. However, the addition of 125 ug/kg biotin in combination with 4% of vegetable oil or a mixture of 2% vegetable oil and 2% tallow in a high Ca diet, appeared to completely overcome the growth depressing effect of a high Ca diet but not of a high Ca and high AP diet. Ca soap formation and Ca inhibition of biotin absorption were postulated to account for the effects of high Ca on fat and biotin absorption. The presence of high AP levels prevented the beneficial effect of vegetable oil and biotin.

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تأثير البيوتين والدهن على أداء واستفادة دجاج اللحم من العناصر الغذائية في التغذية على علائق مختلفة في نسبة الكالسيوم والفسفور

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ملخص البحث. أُجريت تجربتان لدراسة تأثير مستويات مختلفة من الكالسيوم والفسفور والبيوتين والدهن ونوعية الدهن على الأداء وتركيز البيوتين في الكبد، وعلى مدى الاستفادة من مكونات العليقة لدجاج اللحم من عمر ٥-١٩ يوماً.

التغذية على عليقة مرتفعة في الكالسيوم (٢٥,٤، ١٢,٢ جم/كجم) خفض النمو والكفاءة الغذائية لكتاكيت اللحم، بينما عليقة مرتفعة في كلٍ من الكالسيوم والفسفور (٢٥,٧، ١١,٩ مقارنةً بـ ١٢,٢، ٥,٢ جم/كجم) خفضت النمو والكفاءة الغذائية بدرجة أكبر. ووجد أن إضافة ١٢٥ ميكروجراما بيوتين /كجم أو ٤٪ دهون حيوانية أو ٤٪ زيوت نباتية أو خليط من ٢٪ زيوت نباتية، ٢٪ دهون حيوانية لم تساعد في إعادة الوزن المفقود نتيجة التغذية على العليقة المرتفعة في الكالسيوم، بينما إضافة ١٢٥ ميكروجراما بيوتين مع ٤٪ زيوت نباتية أو مع خليط من ٢٪ من كلٍ من زيوت نباتية و دهون حيوانية أعاد الوزن المفقود للكتاكيت المغذاة على عليقة مرتفعة في الكالسيوم وليست للكتاكيت المغذاة على عليقة مرتفعة في الكالسيوم والفسفور .

ويتضح من هذه التجارب أن إضافة البيوتين والزيوت النباتية إلى العليقة المرتفعة في الكالسيوم أدى إلى منع الانخفاض في وزن الكتاكيت.