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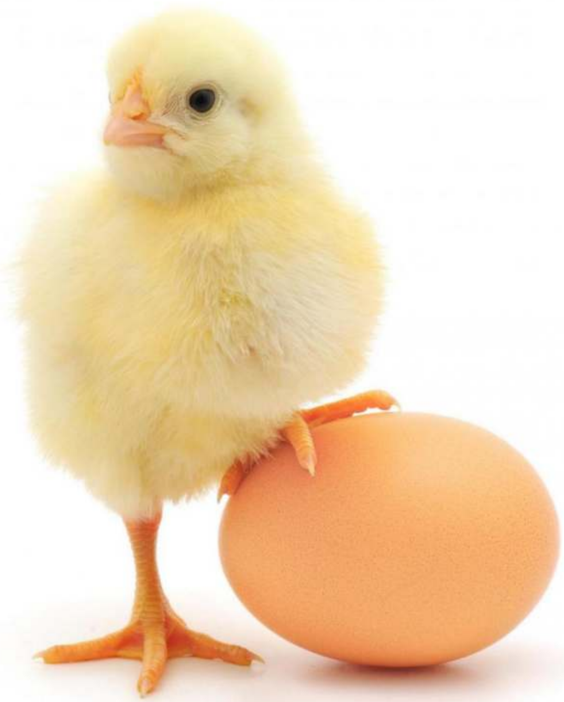

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Effect of Using *Cuminum cyminum* L, Citric Acid and Sodium Sulphate for Improving the Utilization of Low Protein Low Energy Broiler Diets

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Abstract: Three hundred one day-old of unsexed Cobb broiler chicks were used in present study to examine the ability of *Cuminum cyminum* L (CC), Citric Acid (CA) or Sodium Sulphate (SS) either alone or in combination to increase nitrogen retention and utilization of the Low Protein Low Energy Diet (LPLE), containing 4% lower protein and 200 kcal/kg lower energy than control diets. At 42 days of age, LPLE diets decreased weight gain and degrade feed conversion by 23.24% and 19.47%, respectively compared to control diet. Besides, LPLE diet decreased daily nitrogen excretion by 25.92% compared to control diet. Supplementing LPLE diet with CC, CA and SS together improved weight gain, feed conversion and nitrogen retention percentage by 7.21, 6.16 and 16.69%, respectively. Compared to control diet, the combination of such feed additives succeeded in reducing daily nitrogen excretion by 64.81%. It can be conclude that the three additives used in present study work in synergy under low protein and low energy conditions. Further studies are needed to determine the optimum level of these additives mixture with different levels of protein and energy.

Key words: *Cuminum cyminum* L, citric acid, sulphate, low protein energy diet

INTRODUCTION

Intensive animal production is believed to be a major contributor to the pollution of the environment. Among the different sources, agriculture and livestock production represent a major source of emission of both pollutants, i.e. 40% for methane and nitrous oxide and 90% for ammonia (Morard, 2000). On the other hand, excess of nitrogen in manure tend to increase nitrate level in river and ground water. Instances of exceeds of US Environmental Protection Agency limits for nitrate (10 mg/L) in drinking water have been reported in areas where excessive or improper rates of poultry litter have been applied (Ritter and Chirnside, 1982; Minkara *et al*., 1995). El Ramly (1997) showed that the nitrate content of groundwater in Egypt varies from one governorate to another and ranges between 66.45 and 265.8 mg/L. Elevated nitrate concentrations in drinking water are linked to health problems (Wolfe and Patz, 2002). To decrease the nitrate level we must decrease the level of manure and fertilizer. Almasri and Kaluarachchi (2007) concluded that reduction of fertilizer loading was not efficient to decrease nitrate compared to manure loading reduction. A current strategy to reduce the risk of nitrogen pollution of the environment is the reduction of nitrogen excretion in these animal species by dietary manipulation involving the feeding of low-protein, amino acids-supplemented diets. Significant reduction of excreted nitrogen can be achieved by reducing the dietary Crude Protein (CP) levels and balancing the requirements of essential amino acids with synthetic amino acids in poultry (Aletor *et al*., 2000). On the other hand, rate and efficiency of growth is lower and carcass composition becomes inferior in broilers fed diets in which CP has been lowered by more than 3%, even when all known nutrient requirements are met (Fancher and Jensen, 1989; Aletor *et al*., 2000).

All cells of the vertebrate animal depend on a functional antioxidant capacity to provide protection against the harmful effects of free radicals and reactive oxygen species that are the inevitable consequences of aerobic life (Halliwell, 1999). Mitochondria are also a major source of Reactive Oxygen Species (ROS) such as superoxide (O^-_2). The manganese isoform of Superoxide Dismutase (SOD) found in mitochondrial matrix converts O^-_2 to hydrogen peroxide (H_2O_2). Bottje *et al*. (2004) found that the increase in H_2O_2 production and the high protein oxidation were consistently observed in low feed efficiency duodenum, breast muscle and liver mitochondria compared to high feed efficiency birds. Grune *et al*. (1997) showed that the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack. Abd El-Hakim *et al*. (2009) have been used *Curcuma longa* plus thyme as natural antioxidants in broiler diet to decrease the damage of protein by free radical and decrease the lost of protein in feces. They obtained significantly increasing in nitrogen retention by 13.25% compared to control diet and indicated that natural antioxidants may decrease the damage of protein by free radical. They also found that addition of thyme alone or in combination with *Curcuma longa* increased the AME by 3.89 and 3.56%, respectively compared to basal diet.

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The citric acid is known is effective chelate minerals. Nezhad *et al.* (2007) reported that the addition of citric acid to a broiler diet improved feed efficiency. Ali *et al.* (2008) found with rabbit, that the addition of citric acid plus *Curcuma longa* decreased both the harmful microorganisms in the caecum and values of plasma globulin besides they indicated that the combination of these additives saved protein which needed for immunity and directly towards growth.

Cuminum cyminum L (CC) is an annual plant of the Umbelliferae family. This plant, which is one of the important spices in the world, is native to Egypt. Egyptian cumin oil contains 39.2% cuminaldehyde (Srinivas, 1986). The cumin oil, shows anti fungi activity, which could be linked to the cuminaldehyde content (Lawrence, 1992). The use of natural antioxidants in animal nutrition could be restricted due to the low bioavailability of polyphenols. Moreover, many types of polyphenols can lose a part of their antioxidant capacity *in vivo* (Manach *et al*., 2004). Falany (1991) showed that sulfation has evolved as a key step in xenobiotic metabolism. Ali *et al.* (2010) found that the *Cuminum cyminum* L plus sulphate seemed to be the best additive under the heat stress condition. They indicated that addition of sulphate to some extent increase the activity of natural antioxidants. Tonsy *et al.* (2010) showed that mixture of thyme, citric acid and sulphate is the most successful additives for improving performance of Nile tilapia fingers fed low protein diets. This study was conducted to examine the ability of *Cuminum cyminum* L, citric acid or sodium sulphate either alone or in combination to increase the nitrogen retention of the low protein and energy diet (LPLE diet) contain 4% lower protein and 200 kcal/kg lower energy than control diet.

MATERIALS AND METHODS

The experimental work was carried out at El-Takamoly Poultry Project, Research Unit, Fayoum, Egypt while the laboratory work was done at Poultry Nutrition Department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Three hundred one day-old of unsexed Cobb broiler chicks from Misr Alarabia Poultry Company were used in this experiment. They were given a control diet (Table 1) for the first week of age and then chicks were wing-banded, individually weighed and randomly distributed into 10 groups each in three replicates of 10 chicks each and caged in battery brooders. Experimental diets and water were offered *ad-libitum* over the experimental period. Chicks in all treatments were kept under similar conditions of management. Artificial

Table 1: Composition and calculated analysis of the experimental diets

	Control			Low protein			Low protein and energy		
Item	Starter 7-14 d	Grower 15-28 d	Finisher 29-42 d	Starter 7-14 d	Grower 15-28 d	Finisher 29-42 d	Starter 7-14 d	Grower 15-28 d	Finisher 29-42 d
Yellow corn	58.19	61.96	64.00	65.70	73.09	73.57	63.82	70.64	76.10
Soybean meal (44%)	30.12	27.00	23.85	25.60	20.09	17.25	23.70	18.45	16.00
Corn gluten meal (60%)	5.00	3.40	3.68	-	-	-	-	-	-
Soya oil	2.05	3.20	4.19	2.05	1.90	3.40	-	-	-
Lime stone	1.71	1.65	1.55	1.74	1.67	1.58	1.75	1.69	1.58
Mono calcium phosphate	1.70	1.64	1.54	1.75	1.69	1.59	1.68	1.65	1.57
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin and Min. Mix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.15	0.18	0.18	0.32	0.33	0.34	0.32	0.32	0.33
L-Lysine HCl	0.16	0.17	0.21	0.36	0.43	0.47	0.39	0.45	0.49
Sodium bicarbonate	0.27	0.15	0.15	0.27	0.15	0.15	0.27	0.15	0.15
Wheat bran	-	-	-	1.56	-	1.00	7.42	6.00	3.13
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
****Calculated analysis**									
CP (%)	21.06	19.02	18.00	17.07	15.06	14.04	17.00	15.06	14.01
Kcal ME/kg	2988.00	3083.00	3176.00	2988.00	3083.00	3176.00	2788.00	2883.00	2976.00
Crude fiber (%)	3.63	3.46	3.28	3.55	3.14	3.06	4.01	3.63	3.26
Crude fat (%)	4.82	6.03	7.05	4.99	4.97	6.49	3.07	3.20	3.25
Calcium (%)	1.00	0.96	0.90	1.01	0.96	0.90	1.00	0.96	0.90
Available phosphorus (%)	0.50	0.48	0.45	0.506	0.48	0.45	0.50	0.48	0.45
Lysine (%)	1.20	1.11	1.05	1.204	1.10	1.05	1.20	1.10	1.05
Methionine (%)	0.54	0.53	0.52	0.613	0.59	0.59	0.612	0.58	0.58
Methionine + Cystine (%)	0.89	0.84	0.82	0.894	0.84	0.82	0.899	0.84	0.82
Sodium (%)	0.22	0.19	0.19	0.22	0.19	0.19	0.22	0.19	0.19

*Premix contain per 3 kg vit A 12 000 000, vit D3 4000 000 IU, vit E 40 000 mg, Vit K3 7000 mg, vit B1 5500 mg, vit B2 7700 mg, vit B6 6600 mg, vit B12 30 mg, pantothenic acid 18000 mg, Niacin 80000 mg, Biotin 250 mg, Folic acid 1800 mg, Choline chloride 600 gm, Selenium 350 mg, Copper 15000 mg, Iron 110000 mg, Manganese 100000 mg, Zinc 80 000 mg, Iodine 1500 mg, Cobalt 250 mg and $CaCO_3$ to 3000 g. **According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001).

lighting was provided 24 h daily during the whole experimental period. The control diets were supplied with required nutrients to satisfy the recommended requirement of Cobb broilers (Table 1).
Chicks were allotted on the following dietary treatments:

- Control diet.
- Low Protein diets (LP diet) contain 4% lower protein than control diet.
- Low Protein Low Energy diet (LPLE diet) contain 4% lower protein and 200 kcal/kg lower energy than control diet.
- LPLE diet + 0.2% Citric Acid (CA).
- LPLE diet + 0.5% Anhydrous Sodium Sulphate (SS).
- LPLE diet + 0.2% CA + 0.5%SS.
- LPLE diet + 0.2% *Cuminum cyminum* L (CC).
- LPLE diet + 0.2%CC + 0.2%CA.
- LPLE diet + 0.2%CC +0.5%SS.
- LPLE diet + 0.2%CC + 0.2%CA + 0.5%SS.

The LP and LPLE diets were provided by level of lysine and methionine to achieve their corresponding levels of the control diet. Anhydrous Sodium Sulphate was supplied by the Egyptian Salt and Mineral Company. The CC was purchased from local market in Cairo. The citric acid was obtained from Egyptian Company for Laboratory Services, Cairo, Egypt. The Body Weight (BW), Feed Intake (FI) and Body Weight Gain (WG) values were weekly recorded while Feed Conversion (FC) was calculated as a unit of FI per unit of WG. At the end of experimental period (42 days), three birds were taken randomly from each treatment and slaughtered and the edible organs included heart, empty gizzard and liver were weighed. Carcass and organs weights percentage were calculated on the basis of live body weight. Individual blood samples were taken from 3 birds within each treatment and collected into dry clean centrifuge tubes containing drops of heparin and centrifuged for 20 min on (3000 rpm) for obtaining plasma. Antioxidant capacity in plasma was determined using commercial kit produced by Biodiagnostic Company. Plasma cholesterol, total protein, albumin, uric acid and zinc were determined by suitable commercial kits. Globulin concentration of each assayed sample was calculated by subtracting the albumin value from its total protein concentration. The ash retention, nitrogen retention, nitrogen intake (g/day) and nitrogen excreted (g/day) of the tested diets were determined at the end of experiment (42 days) using 3 birds from each treatment throughout digestion trials for 3 days. Feed and the finely ground excreta was analyzed for moisture, ash and Nitrogen content (N) according to official methods (AOAC, 1990). Nitrogen Retention (NR %) was calculated from the following formula. NR = (N content of dry feed-N content of dried excreta) x 100/N content of dry feed. The obtained data were statistically analyzed using analysis of variance procedure (SAS, 1990). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS

Performance: The effect of dietary treatments on body weight gain and feed conversion is shown in Table 2. There were significant differences ($p<0.01$) between values of weight gain in different growth periods while there were insignificant differences between values of feed conversion except for total growth period. In starter period, LP or LPLE diet degraded feed conversion. Although some feed additives used in this study improved feed conversion however, LPLE + SS + CC recorded the worst value (2.03). The addition of CC + CA to LPLE improved feed conversion not only comparable to LPLE but also compared to control diet.
At the end of grower period, the addition of CA improved weight gain and feed conversion by 9.89 and 11.01%, compared to LPLE. Regarding to the finisher period, the addition of mixture of CA + CC + SS to the LPLE diet increased weight gain and improved feed conversion by 9.51 and 2.16%, respectively compared to LPLE diet. All over growth period, the LPLE diet decreased weight gain and degraded feed conversion by 23.24 and 19.47% compared to control diet. In general, it seemed that addition of CA either alone or mixed with CC + SS to the LPLE diet recorded the better feed conversion compared to other additives. The combination of such feed additives improved weight gain and feed conversion by 7.21 and 6.16%, respectively compared to LPLE diet.

Carcass characteristics: The effect of dietary treatments on carcass, liver, heart, gizzard, total edible parts and abdominal fat percentage are shown in Table 3.
No significant differences were detected among treatments for all carcass characteristics except the values of abdominal fat %. The control diet recorded the lowest value of abdominal fat (1.75%) while birds fed LPLE + CC + CA recorded the highest value (3.23%). The addition of CA, CA + SS or CC + CA + SS to LPLE diet numerically decreased the abdominal fat percentage compared to LPLE diet alone.

Ash and nitrogen retention: The data in Table 4 showed that there were significant differences between values of ash retention percentage recorded by dietary treatments. The birds fed LPLE diet plus CA recorded the lowest value while the birds fed LPLE diet supplemented with CA + CC + SS recorded the highest value. The combination of the three feed additives significantly increased ash retention percentage by 25.75% compared to LPLE diet. There were significant differences between nitrogen retention percentage

Table 2: Effect of dietary treatment on growth performance

Treatments	Starter Gain (g)	Starter Feed conversion	Grower Gain (g)	Grower Feed conversion	Finisher Gain (g)	Finisher Feed conversion	Total Gain (g)	Total Feed conversion
Control diet	246.3[a]	1.48	667.0[a]	1.83	1109[a]	2.04	2022[a]	1.90[a]
Low Protein diet (LP)	227.4[ab]	1.72	586.3[b]	2.09	906.8[bc]	2.41	1720[b]	2.21[bcd]
LPLE diet	214.9[b]	1.90	485.6[cde]	2.36	851.8[bc]	2.31	1552[cd]	2.27[bcd]
LPLE diet + CA	226.1[ab]	1.50	533.8[c]	2.10	904.2[bc]	2.19	1671[bc]	2.07[ab]
LPLE diet + SS	219.1[b]	1.63	473.5[de]	2.43	869.5[bc]	2.39	1562[cd]	2.30[cd]
LPLE diet + CA + SS	214.1[b]	1.43	450.2[e]	2.37	840.2[c]	2.35	1515[d]	2.22[bcd]
LPLE diet + CC	210.7[b]	1.86	532.0[c]	2.19	900[bc]	2.33	1656[bc]	2.22[bcd]
LPLE diet + CC+ CA	211.1[b]	1.35	532.8[c]	2.24	901.1[bc]	2.35	1670[bc]	2.19[bcd]
LPLE diet + CC + SS	215.7[b]	2.03	486.0[cde]	2.56	917.5[bc]	2.31	1626[bcd]	2.35[d]
LPLE diet + CC + CA + SS	227[ab]	1.42	509.0[cd]	2.20	932.4[b]	2.26	1664[bc]	2.13[bc]
Pooled MSE	±2.34	±0.06	±6.24	±0.04	±9.58	±0.02	±14.67	±0.02
p-value	0.01	NS	0.0001	NS	0.0001	NS	0.0001	0.004

a,b,..etc.: Means in the same column with different letters, differ significantly (p<0.05). LPLE diet = Low Protein Low Energy diet

Table 3: Effect of dietary treatment on carcass characteristics

Treatments	Carcass (%)	Liver (%)	Gizzard (%)	Heart (%)	Total edible part (%)	Abdominal fat (%)
Control diet	72.14	2.19	1.58	0.62	77.51	1.75[c]
Low Protein diet (LP)	69.55	2.38	1.47	0.62	74.88	3.17[a]
LPLE diet	71.72	2.83	1.71	0.48	77.98	2.45[abc]
LPLE diet + CA	72.25	2.14	1.73	0.53	77.87	2.00[bc]
LPLE diet + SS	70.43	2.28	1.76	0.55	76.24	3.00[ab]
LPLE diet + CA + SS	72.69	2.63	1.54	0.55	78.42	1.80[c]
LPLE diet + CC	72.85	2.08	1.46	0.50	77.85	2.03[bc]
LPLE diet + CC +CA	71.76	2.11	1.67	0.55	77.22	3.23[a]
LPLE diet + CC + SS	71.51	2.59	1.77	0.50	77.65	2.60[abc]
LPLE diet + CC + CA + SS	72.21	2.55	1.47	0.51	77.72	1.87[bc]
Pooled MSE	±0.28	±0.06	±0.03	±0.01	±0.28	±0.13
p-value	NS	NS	NS	NS	NS	0.02

a,b,..etc.: Means in the same column with different letters, differ significantly (p<0.05). LPLE diet = Low Protein Low Energy diet

Table 4: Effect of dietary treatment on ash and nitrogen retention at 42 days

Treatments	Ash retention (%)	Nitrogen intake (g/day)	Nitrogen excreted (g/day)	Nitrogen retention (%)
Control diet	41.27[cd]	3.20	1.08[a]	64.70[c]
Low Protein diet (LP)	43.49[bcd]	2.57	1.00[ab]	60.69[c]
LPLE diet	57.39[b]	2.32	0.80[abc]	66.01[c]
LPLE diet + CA	38.43[d]	2.32	0.86[abc]	63.01[c]
LPLE diet + SS	54.68[bc]	2.30	0.83[abc]	64.24[c]
LPLE diet + CA + SS	57.87[b]	2.41	0.46[bc]	80.57[a]
LPLE diet + CC	56.92[b]	2.26	0.70[abc]	68.88[bc]
LPLE diet + CC + CA	39.02[d]	1.85	0.64[abc]	65.38[c]
LPLE diet + CC + SS	52.31[bcd]	1.68	0.52[bc]	67.74[bc]
LPLE diet + CC + CA + SS	72.17[a]	1.64	0.38[c]	77.03[ab]
Pooled MSE	±2.35	±0.14	±0.05	±1.35
p-value	0.0020	NS	0.04	0.016

a,b,..etc.: Means in the same column with different letters, differ significantly p<0.05. LPLE diet = Low Protein Low Energy diet

values recorded by different dietary treatments (Table 4). The low level of energy and protein in LPLE diet decreased daily nitrogen excretion by 25.92% compared to control diet.

The addition of CA + SS to LPLE diet significantly increased nitrogen retention by 22.05% compared to LPLE diet. However, when the three additives added to LPLE diet, the nitrogen retention percentage increased by 16.69% compared to LPLE diet.

Plasma parameters: The data in Table 5 showed that there were significant differences between plasma cholesterol values recorded by different dietary treatments without consistent trend. There were significant differences among treatments in values of plasma total protein and globulin while, there were insignificant differences between albumin values. It was surprise that LPLE diet recorded the highest value of plasma total protein (3.02 g/dl) and globulin (2.31 g/dl).

Table 5: Effect of dietary treatments on some plasma parameters

Treatments	Total cholesterol (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Uric acid (mg/dl)	Zinc (μg/dl)	Antioxidants capacity (mmol/L)
Control diet	95.85^{bc}	2.77^{ab}	1.03	1.74^{ab}	1.19^{cde}	703.2^{a}	0.56^{c}
Low protein diet (LP)	108.08^{bc}	1.63^{cd}	0.96	0.67^{bc}	0.97^{de}	705.2^{a}	0.94^{a}
LPLE diet	108.08^{bc}	3.02^{a}	0.71	2.31^{a}	0.78^{e}	674.1^{ab}	0.66^{bc}
LPLE diet + CA	102.16^{bc}	1.43^{d}	0.81	0.75^{bc}	1.39^{cd}	708.2^{a}	0.68^{bc}
LPLE diet + SS	98.48^{bc}	1.35^{d}	1.01	0.34^{c}	1.53^{c}	711.1^{a}	0.31^{d}
LPLE diet + CA + SS	79.68^{c}	2.28^{abcd}	1.34	0.94^{bc}	1.65^{c}	709.4^{a}	0.94^{a}
LPLE diet + CC	118.47^{ab}	2.57^{abc}	0.85	1.72^{ab}	2.12^{b}	703.5^{a}	0.82^{ab}
LPLE diet + CC + CA	144.51^{a}	1.90^{bcd}	0.89	1.01^{bc}	3.62^{a}	676.6^{ab}	0.89^{a}
LPLE diet + CC + SS	125.94^{ab}	1.77^{bcd}	0.74	1.02^{bc}	1.57^{c}	654.5^{bc}	0.64^{bc}
LPLE diet + CC+ CA+SS	113.08^{abc}	2.62^{abc}	1.12	1.49^{ab}	1.57^{c}	636.6^{c}	0.53^{c}
Pooled MSE	±4.23	±0.13	0.04	±0.11	±0.14	±5.49	±0.03
p-value	0.03	0.007	NS	0.01	0.0001	0.0007	0.0001

a,b,..etc.: Means in the same column with different letters, differ significantly $p<0.05$. LPLE diet = Low Protein Low Energy diet

The addition of CC + CA significantly decreased plasma globulin by 56.27% compared to LPLE diet. There were significant differences among plasma uric acid levels due to different dietary treatments. The birds fed LPLE diet recorded value lowered plasma uric acid by 34.45% compared to control diet while, the birds fed LPLE diet + CC + CA recorded the highest value (3.26 mg/dl) (Table 4). The addition of the three additives (CC + CA + SS) decreased the plasma uric acid compared to CC + CA. The analysis of variance indicated that there were significant differences between plasma zinc values. The addition of the mixture of three feed additives (CC + CA + SS) used in this study to LPLE diet recorded the lowest value (636.6 μg/dl) of plasma zinc. There were significant differences between plasma antioxidants capacity values. LP diet recorded significantly higher value than control diet. The addition of CC either alone or with CA to LPLE diet increased total plasma antioxidants capacity compared to LPLE diet. While the addition of CA + SS to LPLE diet significantly increased plasma antioxidants by 42.42%, addition of SS either alone or with CC decreased it, compared to LPLE diet.

DISCUSSION

In the present study, the effect of three feed additives and their combination in increasing the utilization of low protein low energy diets is the main target.

The use of LP or LPLE diets decreased weight gain and degraded feed conversion, these results agree with those obtained by Aletor *et al.* (2000) who found that the increased feed consumption in the low-protein diets led to a corresponding inferior feed conversion efficiency. The beneficial effect of CC + CA in starter period have been observed by Ali *et al.* (2008) who found with rabbit that the addition of citric acid plus Turmeric act to save protein which needed for immunity and directly towards growth. Also, there are some studies reporting that acidified feed plus herb extract give good results. For example, Spais *et al.* (2002) found that a commercial feed additive containing herb extracts and organic acids exerts a growth promoting effect comparable to that of flavomycin. Lippens *et al.* (2005) showed that organic acids and plant extracts work differently while, the combination of both groups of products could give an additive effect. At the end of grower period, addition of CA improved weight gain and feed conversion.

It is probable that supplementation of CA has a beneficial effect under LPLE condition. It is well known that several amino acids form chelates with polyvalent cations and there is a strong evidence that these chelates aid in the passage of the metal ions across cell membranes (Scott *et al.*, 1982). For example, dietary protein has been shown an effect on absorption and retention of zinc, copper and iron in rats (Van Campen and House, 1974). Adding CA may increase the retention of trace minerals which may decrease as a result of using low protein diets. The retention of trace minerals is very important for their role in antioxidant enzymes defense. For example, Manganese (Mn) is a crucial component of the metalloenzyme (Mn Superoxide Dismutase, MnSOD). Luo *et al.* (1992) determined that MnSOD functioning as a free radical scavenger is by far the most important Mn-containing enzyme. Also, one of the most significant functions of zinc is related to its antioxidant role and its participation in the antioxidant defense system (Powell, 2000). On the other hand, mixture of three feed additive recorded the highest weight gain value in finisher period. Hypothesis to explain the beneficial effect of SS when added to CC + CA is that sulphate may increase the activity of natural antioxidants (Ali *et al.*, 2007) and/or increase the mineral retention. In this respect, Ali *et al.* (2010) found that sulphate addition increased ash retention by 61.63% compared to control diet in growing chicks under heat stress condition. As mentioned before, under low protein condition, the trace elements retention may decrease and addition of CA + SS may play a role in increasing such elements retention while CC decrease the bad effect of free radical on protein degradation. The beneficial effect of mixture of feed additives under low protein diet have been observed before by Tonsy *et al.* (2010) who found that the mixture of thyme, citric acid and sulphate is the most successful additives for improving performance of Nile tilapia fingerlings fed low protein diets.

The increase of abdominal fat in groups fed LPLE either alone or with some additives (SS or CC + CA) can be explained on the basis that low protein diet promote lipid synthesis. Donaldson (1985) showed that low-protein diets promote higher rates of *de novo* hepatic lipid synthesis in chickens than high protein diets. The addition of CA, CA + SS or CC + CA + SS to LPLE diet numerically decreased the abdominal fat percentage compared to LPLE diet indicating that these additives may save the protein and consequently decrease fat deposition.

The birds fed LPLE diet supplemented with CA + CC + SS recorded the highest value of ash retention. As mentioned before, the lower level of protein was accompanied with lower trace mineral retention since the dietary protein has been shown to affect the absorption and retention of zinc, copper and iron (Van Campen and House, 1974). Therefore, it is preferable to add some feed additives to increase the availability and retention of these trace minerals under low protein diet. In the present study, we added CA to increase availability of some trace mineral. For example, Boling *et al.* (2001) reported in chickens that the Zn utilization increased by the addition of CA to diet. Also, Sugiura *et al.* (1998) observed an increase in the apparent availability of calcium, phosphorus, magnesium, manganese and iron in rainbow trout fed fish meal based diets supplemented with CA. Furthermore, it is known that minerals in sulphate form have higher availability values than others. Keeping in consideration that some of these trace minerals like iron or copper in the free form become prooxidant (Ercal *et al.*, 2001) but, in binding from with protein, it become safe thus trace elements need also protein in the cell and the later may be saved by natural antioxidants like CC. From previous discussion, it is easy to explain the reason of beneficial effect of combination of three feed additives on ash retention. The results agree with those found by Tonsy *et al.* (2010) who found with fish fed low protein diet that the mixture of thyme, citric acid and sulphate significantly increased ash percentage in fish meat by 12.21% compared to basal diet. The reduction in daily nitrogen excretion of birds fed LPLE diet (25.92%) agree with those obtained by Bregendahl *et al.* (2002) who found that chicks fed low-protein diets excreted less nitrogen than those chicks fed the high-protein diets and nitrogen excretion increased linearly with nitrogen intake. Yamazaki *et al.* (2007) concluded that the CP content of a broiler diet can be reduced by 20 g/kg diet and nitrogen excretion is thereby reduced by about 25%. Corzo *et al.* (2005) found the highest percentage retention and lowest excretion of nitrogen in birds fed the low crude protein diet (40 g/kg diet). The addition of CA + SS to LPLE increased nitrogen retention percentage by 22.05% and these results can be explained on the basis that CA + SS may increase the trace minerals retention which help birds to decrease free radicals that attack the protein. In this respect, Grune *et al.* (1997) showed that the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack. Iqbal *et al.* (2004) showed that if reactive oxygen species did not removed by antioxidants then oxidation of critical structures in the mitochondria or cell or both, such as lipids, proteins and DNA, can lead to further inefficiencies that accentuate additional reactive oxygen species generation. For these reasons, the addition of CA + SS to the LPLE diet increased nitrogen retention percentage but the data of performance in Table 2 showed that this treatment is not better as we expected. One hypothesis to explain the increase of nitrogen retention percentage with this combination of feed additives without improving performance is that CA plus SS can protect protein by increasing trace mineral retention and consequently increase antioxidants enzyme since tissue antioxidant protection is mostly enzymatic rather than micro molecular (Cohen *et al.*, 2007) but the lipid may need special antioxidants like compounds which dissolve in lipid present in CC. For instance, water-soluble antioxidants such as ascorbic acid intercept free radicals in the aqueous phase (Meister, 1992), while vitamin E, located within the lipid bilayer of cellular membranes, breaks the chain reactions of fatty acid peroxidation (Brigelius-Flohe and Traber, 1999).

For these reasons, when the three additives added to LPLE diet, both nitrogen retention percentage and performance improved. However, Sen *et al.* (1997) suggested that different mechanisms or factors possibly influence oxidative damage to lipids and proteins. The increase in both ash and nitrogen retention percentage when the LPLE was supplemented with CA + CC + SS may due to that firstly, CC plays a role as an antioxidants to protect lipids from free radical attack and consequently decrease the degradation of protein. Secondly, CA plays a role to increase the mineral retention which expected to be decreased under low protein diet thirdly, sulphate increase the activity of antioxidants and/or increase trace mineral retention. In this respect, Surai (2002) showed that to achieve optimum protection, the tissues deploy an integrated antioxidant system that consists of a diverse array of lipid-soluble (e.g. vitamin E, carotenoids), water-soluble (e.g. ascorbic acid, glutathione) and enzyme (e.g. glutathione peroxidase, superoxide dismutase) components. The key feature is that these various components act in synergy. Based on the previous consideration, we can explain the highest reduction in daily nitrogen excreted (64.81%) with mixture of the three feed additives compared to control diet. Regarding to performance data in Table 2 and nitrogen retention in Table 4, we can conclude that the three additives CC + CA + SS) work in synergy under LPLE diet condition.

The reduction in plasma globulin in birds fed LPLE + CC + CA have been observed before by Ali *et al.* (2008) who found with rabbit that the addition of CA plus Turmeric decreased both the harmful microorganisms in the caecum and values of plasma globulin. Richards *et al.* (2005) showed that microflora-specific immunoglobulin A and immunoglobulin G secretion alone can cost the animal several hundred grams of protein over a lifetime that is not directed towards growth. Le Floc'h *et al.* (2004) showed that stimulation of the immune system disturbs normal body processes and in turn is able to induce specific amino acid requirements. The difference between LP and LPLE diet in globulin values can be explained on the basis that LP diet contained soy bean oil which plays a role in protection of the feed passage in digestive tract from bacteria attack since fatty acids have been identified as bactericidal factors (Canas-Roderiqeuz and Smith, 1966). Based on results observed in this study, we can indicate that these feed additives may save the protein by protecting the protein from free radical and/or decrease protein consumed in immune globulin synthesis (immune cost). The reduction in plasma uric acid of birds fed LPLE agree with those of Corzo *et al.* (2005) who found that control diet (22% CP) significantly increased uric acid concentration compared to low protein diet (18% CP) and suggested that this increase means that an excess of nitrogen compounds are needed to eliminate. The addition of CC + CA increase plasma uric acid and these results indicated that these birds have an excess of nitrogen compounds. We have shown previously that, the addition of CC + CA may act synergy to inhibit bacteria population and consequently decrease immune cost and save the protein and/or protect protein from free radical attack and this saving in protein occur also in daily nitrogen excretion. The addition of the three additives (CC + CA + SS) decreased the plasma uric acid compared to CC + CA meaning that excess of nitrogen compounds are utilized by birds and this occur in nitrogen retention percentage and excretion (Table 4). It seemed that data of nitrogen retention percentage and excretion are in harmony with plasma total protein and uric acid. LP diets contain much oil compared to others which may increase the availability of antioxidants compounds soluble in lipid and consequently increased plasma antioxidants capacity (Table 5). However, at the cellular level, hydrophilic antioxidants are found in the cytoplasm, whereas lipophilic antioxidants are found in cell membranes (Halliwell and Gutteridge, 2006).

The addition of CC either alone or with CA increased plasma antioxidants capacity. These results agree with those obtained by Ali *et al.* (2007) who found that addition of anise or thyme increased plasma antioxidants capacity of laying hens. On the other hand, CA + SS work synergy to protect protein from free radical and this occur in nitrogen retention percentage (Table 4) and consequently increased antioxidants capacity since the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack (Grune *et al.*, 1997). Also, xenobiotic conjugation with sulphate is an important route for conversion of lipophilic xenobiotics to more readily excreted polar metabolites (Jakoby, 1980). The SS conjugate with phenolic compounds and/or other xenobiotics present in feed ingredients or CC may convert it from lipid soluble to water soluble. However, circulating antioxidants might or might not correlate with enzymatic antioxidant levels in tissues (Cohen *et al.* 2007). For this reason, we expected that addition of SS increased availability of these natural antioxidants and move it to cells and not important to occur in total plasma antioxidants capacity.

Conclusion: Using LPLE diet decreased broiler weight gain, degraded feed conversion by 23.24 and 19.47% and decreased daily nitrogen excretion by 25.92% compared to control diet. The addition of mixture of the three feed additives (CC + CA + SS) improved weight gain, feed conversion and nitrogen retention percentage by 7.21, 6.16 and 16.69%, respectively compared to LPLE diet. Comparing to control diet, the mixture of the additives reduced daily nitrogen excreted by 64.81%. It can be concluded that the tested three additives used in this study work in synergy under extreme low protein diet (40 g/kg). Further studies are needed to determine the optimum level of these additives with different levels of protein and energy.

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