Full Length Research Paper

Dietary fortification of a natural biosurfactant, lysolecithin in broiler

T. Melegy, N. F. Khaled*, R. El-Bana and H. Abdellatif

Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, 12211, Giza, Egypt.

Accepted 27 September, 2010

Responses to dietary fortification of natural biosurfactant were investigated using broiler performance. serum chemistry and carcass traits. A total of 996 day old broiler chicks (Cobb 500 strain) were weighed individually and randomly assigned into four groups and three replicates per group with 83 birds per replicate. Group I, (negative control) was fed a lower nutrient density test diet without Lysoforte Booster® (lysolecithin). While, group II, (positive control) was fed the basal diet (corn-soyabean meal based), recommended by breed catalogue without Lysoforte Booster®. However, groups III and IV were fed the negative control diet with Lysoforte Booster® at a rate of 250 and 500 g/ton of feed on top, respectively. Results showed a significant (P < 0.05) lowest final weight, lowest weight gain, poorest FCR and highest feed intake in group I in comparison to the positive control and the Lysoforte Booster® supplemented groups. The negative control also had a significant (P < 0.05) higher mortality rate compared to the Lysoforte Booster® supplemented groups. Serum metabolic profile was not significant affected by dietary fortification with lysoforte booster. There was a significant (P < 0.05) increase in dressing percent in groups supplemented with Lysoforte Booster® compared to the negative control group. However, liver indices in the Lysoforte Booster® treatments were significantly (P < 0.01) lower than the positive control group. It could be concluded that the dietary fortification of lysoforte booster can improve the broiler productivity, modify carcass quality and formulate more cheaper diets with reduced energy and amino acids without any adverse effect on broilers overall performance.

Key words: Broiler, lysoforte booster, performance, serum, carcass.

INTRODUCTION

Fat is mainly composed of triglycerides. The problem of fat digestion is that this process takes place in an aqueous environment, as in gastrointestinal tract, although fats are not water-soluble. Fat is emulsified by detergent action of the bile salts and hydrolysed by lipase into fatty acids and mono- and diglycerides. Transport of fat and mono- and diglycerides occurs in the form of micelles: Therefore, biosurfactants are needed such as phospholipids, lecithins and lysolecithins (Polin, 1980; Soares and Lopez-Bote, 2002).

The physiological ability of young birds for fat utilization is poorly developed and a marked improvement of the Apparent Metabolizable Energy (AME) value of fats has

been reported in birds that are 1.5 to 3.5 weeks of age (Freeman, 1984; Wisman and Salvador, 1989).

Soy lysolecithin is an excellent emulsifier for food and has been prepared by pancreatic phospholipase A_2 -catalyzed hydrolysis of soy lecithin. The emulsion with soy lysolecithin is stable in various conditions, for example high temperature, acidic solution and high salt concentration. Soy lysolecithin is also a good solubilizer. There is a sort of interaction between soy lysolecithin and dietary protein, a situation that may affect protein absorption and utilization (Aoi, 1990).

The use of supplemental fats and oils in broiler chicken diets as an energy source has become a wide-spread practice in the feed industry. There is a shortage of some digestion enzymes in young birds and fat digestion improves with age (Hertrampf, 2001).

Lysoforte Booster® is a specific natural phospholipid, prepared commercially from soya lecithin that proved to

^{*}Corresponding author. E-mail: fahmy66@hotmail.com. Tel: +20182009187 or +2028329276. Fax: +2025725240.

have the ability to act as an absorption enhancer in poultry and animal. Dietary fortification of a natural biosurfactant (Lysoforte Booster) assists and promotes the absorption of different nutrients (Schwarzer and Adams, 1996).

Phospholipids have a significant function in the metabolism of animals, particularly in lipid metabolism. In general, plant feedstuffs do not contain high amounts of phospholipids, except for soybeans. Soy lecithin may be used either directly by incorporating soybeans in animal diets or by incorporating a concentrate (Liu et al., 2003; Wang et al., 2004).

On the other hand, the utilization of phospholipids as an energy source has received little attention in poultry nutrition despite its impact on improving lipid metabolism and also the studies investigating the effect of dietary phospholipids on performance of chicken and young pigs are scarce and limited (Azman and Ciftci, 2004; Attia et al., 2008).

This study was designed to study the effect of dietary inclusion of Lysofore Booster[®] (Lysophosphatidyle choline, recognized as a natural absorption enhancer) on growth performance, serum chemistry and carcass traits in broilers.

MATERIALS AND METHODS

Lysoforte Booster[®] that is composed mainly of lysophosphatidyle choline (Lysolecithin), recognized as a natural absorption enhancer and manufactured by Kemin Europa, Herentals, Belgium, was included in a day-old broiler chick (Cobb 500 strain) diets (starter and grower- finisher) at the rate of 250 g or 500 g/ton of feed for 6 weeks in order to investigate the effects of dietary fortification of lysolecithin on performance parameters, serum indices and carcass traits.

Birds and diets

A total of 996 day old broiler chicks (Cobb 500 strain) were weighed individually and randomly assigned into four groups and three replicate per group with 83 birds per replicate:

Group I (Negative control)

This group was fed lower nutrient density test diet without Lysoforte Booster[®]. Diets were corn-soyabean meal based and were lower in added oil and in synthetic amino acids (L-Lysine and DL-methionine).

Group II (Positive control)

This group was fed basal diet recommended by breed catalogue without Lysoforte Booster[®]. Diets were corn-soyabean meal based.

Group III

This group was fed negative control diet with Lysoforte Booster[®] at a rate of 250 g/ton of feed on top from day one until slaughter.

Group IV

This group was fed negative control diet with Lysoforte Booster[®] at a rate of 500 g/ton on top from day one until slaughter.

Chicks were floor reared in an electrically heated experimental room bedded by a layer of wood shavings, with a constant lighting program employed during the whole experimental period. The birds were provided with clean water and fed *ad-libitum* on the starter diet for the first three weeks and on grower-finisher diet for the last three weeks. Birds were kept under standard hygienic conditions and were subjected to a prophylactic vaccination and pharmacological program against viral and bacterial diseases.

The basal diets were formulated to meet all the nutrient requirements of the Cobb 500 strain of broilers according to the recommendations established by the breed producers. Composition, calculated and chemical analysis of different diets according to AOAC (1990) are illustrated in Table 1. Diets were formulated by using UNE Form software linear programming (1999). Diets were calculated based on the nutrient composition for the feeds published by the Central Lab for Food and Feed (CLFF), Ministry of Agriculture, Agricultural Research center, Giza, Egypt (Technical Bulletin Nr.1, 2001). Reduction of soya oil, DL-Methionine and L-Lysine was replaced by Lysoforte Booster® based on the recommendation from the manufacturing company.

This study was carried out at the Animal and Poultry Research Center of the Faculty of Veterinary Medicine, Cairo University, Egypt.

Measurements

The growth performance data

The growth performance of broiler chickens were evaluated in terms of body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR). Individual BWG of the birds were recorded at the beginning of the experiment and on a weekly basis thereafter. Weekly records of FC for each group were also maintained in order to calculate FCR (g feed: g gain).

Serum biochemical analysis

The blood samples were individually collected from the wing vein of ten birds per replicate in the middle and end of the study. Serum was separated and frozen at $-20^{\circ}\mathrm{C}$ until it was assayed. Determination of serum triglycerides (Fossati and Prencipe, 1982), cholesterol (Allain et al., 1974), total lipids (Zollner and Kirsch, 1962), uric acid (Fossati et al., 1980), protein (Gornal et al., 1949), creatinine (Fabiny and Ertingshausen, 1971), AST (Reitman and Frankel, 1957), ALP (Roy, 1970) and ALT (Reitman and Frankel, 1957) were carried out using photometric methods and diagnostic kits (Biodiagnostic, Egypt).

Carcass traits

At the end of the experimental period, five birds from each replicate in both control and experimental groups were randomly chosen and were left overnight in the waiting yard where only water was allowed. Each bird was weighed then hanged, slaughtered, scalded at 55 to 65°C, de-feathered, eviscerated and washed with tap water. The carcass was then placed on a processing table where the breast meat (deboned breast meat yield without skin) was cut from the remaining upper back and rib cage of the carcass, washed, cooled in ice water tank for two hours, dried for ten minutes, and the dressing yield % (DY%), breast muscle yield

Table 1. Composition and chemical analysis of basal and experimental diets.

In ave die na	Co	ntrol diets	Experimental diets		
Ingredient	Starter	Grower-finisher	Starter	Grower-finisher	
Yellow corn	55.78	60.40	56.57	61.23	
Soybean meal (44%CP)	29.19	23.83	29.19	23.83	
Corn gluten meal(60% CP)	7.50	7.50	7.50	7.50	
Soy oil	3.04	4.10	2.29	3.35	
Common Salt (NaCl)	0.35	0.35	0.35	0.35	
Sodium Bicarbonate	0.04	0.06	0.04	0.06	
L-Lysine	0.20	0.18	0.15	0.13	
DL-Methionine	0.06	0.07	0.04	0.05	
Monocalcium phosphate	1.76	1.55	1.76	1.55	
Limestone	1.81	1.66	1.81	1.66	
Broiler premix*	0.30	0.30	0.30	0.30	
Total	100	100	100	100	
Calculated analysis					
ME Kcal/kg	3050.00	3175.00	3015.00	3140.00	
CP %	22.00	20.00	22.00	20.00	
EE %	6.33	7.35	5.61	6.63	
CF %	3.45	3.15	3.45	3.15	
Lysine %	1.28	1.10	1.24	1.06	
Methionine %	0.50	0.48	0.48	0.46	
Met+Cys %	0.89	0.84	0.87	0.82	
Ca %	1.00	0.90	1.00	0.90	
P (total) %	0.81	0.74	0.81	0.74	
P (available) %	0.50	0.45	0.50	0.45	
Ca/P ratio	2.00	2.00	2.00	2.00	
Chemical analysis					
CP %	22.33	20.13	22.30	20.10	
EE %	6.52	7.43	5.5	6.54	
CF %	3.57	3.4	3.57	3.4	
Ca %	1.1	1.00	1.1	1.00	
P (total) %	0.70	0.69	0.70	0.69	

Broiler premix* Each 3 kg of vitamin and mineral mixture contains: 13000000 IU vitamin A; 5000000 IU D₃; 80000 mg E; 4000 K mg; 5000 mg B_1 ; 9000 mg B_2 ; 4000 mg B_6 ; 20 mg B_{12} ; 15000 mg pantothenic acid; 60000 mg Nicotinic acid; 2000 mg Folic acid ; 150 mg Biotin ; 400000 mg Choline Chloride ; 20000 mg Copper sulphate ; 1000 mg calcium lodide ; 50000 mg ferrous sulphate; 100000 mg Manganese oxide; 100000 mg Zinc oxide and 300 mg Selenium selenite; Lysoforte Booster® was added at a rate of 250 and 500 g /ton on top of one ton of group (3) and group (4) respectively on starter and grower-finisher experimental diets.

(BMY%) and thigh yield (TY%) were recorded according to El-Banna et al. (2003). The weight of liver, spleen, bursa and heart weight for each bird was recorded, and then the indices were calculated.

Statistical analysis

Data for all variables were subjected to analyses of variance (ANOVA)in order to assess the effect of inclusion levels of Lysoforte booster using the general linear models procedure in SPSS® statistical softw are (SPSS, 2006). Statistical significance was accepted at P < 0.05.

RESULTS AND DISCUSSION

Growth performance parameters

Data concerning the growth performance parameters (weight gain, feed consumption and feed conversion ratios) are presented in Table 2. Results revealed that birds in a negative control group that fed on a lower nutrient density diets only (lower in added oil and in synthetic amino acids) without Lysoforte Booster® showed a significant (P < 0.05) lowest final body weight,

Table 2. The effect of dietary fortification of lysoforte booster on growth performance of broilers.

Parameter	Negative control	Positive control	Negative control + 250g Lysoforte Booster	Negative control + 500g Lysoforte Booster	P value
No. of chicks	239	239	240	242	
Final body weight (g)	2238.69 ^b ± 49.9	2312.72 ^a ± 39.7	2338.51 ^a ± 37.4	2302.09 ^a ± 45.1	0.019
Total gain (g)	2176.83 ^b ± 50.1	2262.55 ^a ± 40.1	2289.26 ^a ± 37.4	2252.17 ^a ± 44.3	0.018
Total feed consumption (g/chick)	4577.3 ^a ± 10.6	4526.94 ^b ± 8.43	4542.28 ^{ab} ± 47.2	4521 ^{ab} ± 46.7	0.003
Average FCR	$2.1^a \pm 0.06$	$2.00^{ab} \pm 0.05$	$1.98^{b} \pm 0.05$	$2.00^{ab} \pm 0.01$	0.028
Mortality%	$4.02^a \pm 0.30$	$4.02^a \pm 0.30$	3.61 ^b ± 0.25	2.81° ± 0.22	0.008

^{ab}Means within the same raw with different superscripts are significantly different at P < 0.05; Values are means ± SD.

lowest weight gain, poorest FCR and highest feed consumption in comparison to the positive control and the Lysoforte Booster[®] supplemented groups.

The negative control also had a significant (P < 0.05) higher mortality rate compared to the Lysoforte Booster® supplemented groups. However, no significant differences were detected in the same parameters among other groups. These findings can be attributed to that Lysoforte Booster® supplementation as the absorption enhancer has enhanced the digestion of fat by facilitating its emulsification with better fat absorption. Lysoforte Booster® has increased energy utilization and assisted in the absorption of other soluble nutrients and vitamins.

However, our findings are in agreement with those of Jones et al. (1992) who observed that emulsification of fat may improve fat digestibility and growth performance of weaned pigs fed supplemental fat, the same authors reported an increase in fat digestibility when lecithin or lysolecithin were added to nursery diets containing soybean oil or tallow, but not in diets containing lard. In addition, Averette (2001) reported that inclusion levels of dietary lysine can be reduced using lysolecithins and it has been hypothesized that improved homogenisation of the feed by lysolipids, results in enhanced digestibility of many water-soluble nutrients.

The feeding of an emulsifier (Lysoforte Booster®) as a natural absorption enhancer resulted in similar responses to the control diet. On the contrary, reducing dietary amino acids and energy without feeding the natural absorption enhancer resulted in poorer performance. The compensatory action of lysolecithins may be attributed to its unique mode of action in absorption enhancement as Inoue et al. (1977) mentioned that lysolecithins arrange themselves into micelles and Liposomes. The smaller the micelle, the easier it crosses the cell membrane as stated by Reynier et al. (1985). The lysolecithins have only one fatty acid resulting in "close packing" ultimately producing smaller and better micelles for diffusion across the cell walls. These micelles and liposomes fuse into the membrane of the gut releasing their contents into the blood hence aiding lipid absorption. The liposome itself

has an advantage over the micelle in carrying both fat soluble nutrients and water soluble nutrients and since the lysolecithins have the ability to form liposomes, hence, lysolecithins plays a vital role in increasing protein solubility, absorption and synthesis and this conversation is in agreement with O'Doherty et al. (1973), who stated that phospholipids stimulate protein synthesis during active fat absorption and Xing et al. (2004) who stated that lysolecithins improve digestibility of fat as well as protein.

A second feature of the action of lysolecithins as absorption enhancer is that individual lysolecithins enter into the gut membrane, In doing so, they increase the porosity of the membrane encouraging nutrients in the lumen of the gut across the membrane and into the blood, as Khidir et al. (1995) stated that lower concentrations of lysophosphatidyle choline permeabilize the cell surface membranes, Weltzien (1979) stated that lysophosphatidyle choline replaces phosphatidyle choline in the membrane bilayer.

The energy sparing effect of lysolecithin may be attributed to its ability to form micelles and liposomes, hence, it spares the action for excess bile synthesis through enhanced fat digestion. This was confirmed by Lennox et al. (1968) who stated that lysolecithin is a more effective emulsifier than bile salts, and also by Attia et al. (2008) who attributed the improvement in laying performance of hens to the extra metabolizable energy obtained from soy phospholipids being added to their diets and considered soy phospholipids as an extra metabolizable energy sources, which can increase the energy availability.

The serum metabolic profile

The blood metabolic profile, primarily used to detect subclinical disorders as a result of malnutrition, has recently been used more widely to evaluate the effects of different dietary fortifications on metabolic, nutritional and welfare conditions of animals (Bertoni et al., 2000; Bovera et al., 2007).

Serum parameters as a picture of metabolic profile at

Table 3. Effects of dietary fortifications of lysoforte booster on serum biochemical indices.

Parameter	Negative control	Positive control	Negative control + 250 g Lysoforte Booster	Negative control + 500 g Lysoforte Booster	P value	
Total protein (g/dl)						
21 d	3.27 ± 0.12	3.37 ± 0.2	3.40 ± 0.4	3.31 ± 0.3	0.21	
40 d	3.67 ± 0.2	3.51 ± 0.2	3.63 ± 0.2	3.48 ± 0.3	0.73	
ALT (units/ml)						
21 d	34.33 ± 1.3	35.00 ± 2.2	34.33 ± 1.3	35.33 ± 1.3	0.43	
40 d	35.67 ± 0.5	35.67 ± 0.5	36.00 ± 0.8	35.67 ± 0.9	0.22	
AST (units/ml)						
21 d	93.50 ± 5.3	95.58 ± 5.1	94.25 ± 4.4	94.00 ± 4.9	0.13	
40 d	103.5 ± 0.5	100.7± 9.9	103 ± 10.2	97.58 ± 9.3	0.26	
ALP (units/ml)						
21 d	67.5 ± 26.2	75.6 ± 12.8	54.2 ± 22.6	62.00 ± 20.1	0.74	
40 d	67.2 ± 25.6	75 ± 14.7	58.2 ± 21.3	64.75 ± 23.9	0.41	
Uric acid (mg/dl)						
21 d	6.65 ± 1.24	7.35 ± 0.4	8.40 ± 0.4	7.13 ± 1.3	0.10	
40 d	3.67 ± 0.9	3.51 ± 0.9	3.63 ± 1.9	3.48 ± 1.8	0.19	
Creatinine (mg/dl)						
21 d	0.29 ± 0.13	0.35 ± 0.04	0.38 ± 0.1	0.34 ± 0.1	0.51	
40 d	0.37 ± 0.05	0.39 ± 0.1	0.52 ± 0.11	0.36 ± 0.14	0.12	
Triglyceride(mg/dl)						
21 d	90.00 ± 7.6	91.75 ± 2.8	80.58 ± 5.6	85.33 ± 4.5	0.07	
40 d	96.33 ± 2.4	93.00 ± 4.1	87.42 ± 9.1	86.50 ± 3.1	0.07	
Cholesterol (mg/dl)						
21 d	122 ±12.6	115 ± 10.8	110 ± 14.1	117 ± 12.9	0.65	
40 d	124.3 ± 3.7	126 ± 9.4	119.5 ± 9.8	115 ±9.1	0.28	

Values are means ± SD; * Non significant.

21 d and 40 d are presented in Table 3. No significant (P ≤ 0.05) differences were observed between all groups; a situation, which indicates that there were no alterations in the liver and kidney and other internal organs functions. The obtained data revealed that the serum metabolic profile, such as total proteins, AST and ALT were not significantly affected by Lysoforte Booster[®] fortification. This indicates that protein metabolism and liver function were not adversely affected by different levels of supplemented Lysoforte Booster, and this might be due to high availability of dietary CP/amino acid and the effect of soy phoshpolipids on improving liver functions (Attia et al., 2008).

Meanwhile, cholesterol and triglycerides were not significantly affected by soy phospholipid supplementation. In this regard, An et al. (1997) reported that cholesterol,

high-density lipoprotein, triglyceride and phospholipid were not significantly affected by phospholipid supplementation from either safflower crude or purified phospholipid and safflower oil. In contrast, Jones et al. (1992) stated that the feeding of lysolecithin (Lysoforte Booster) tended to lower serum triglycerides, while, serum non-esterfied fatty acids were not affected.

Carcass traits

Data of the dressing percent, breast muscle yield (BMY), thigh yield (TY) and indices of different internal organs at the end of experiment are presented in Table 4. Results revealed that there was a significant (P < 0.05) increase in dressing percent in groups supplemented with

Table 4. Effects of dietary fortifications of lysoforte booster on carcass traits of broilers.

Parameter (%)	Negative control	Positive control	Negative control + 250 g Lysoforte Booster	Negative control + 500 g Lysoforte Booster	P value
Dressing	75.66 ^b ± 1.0	$76.12^{ab} \pm 3.0$	76.78 ^a ± 1.4	77.01 ^a ± 1.9	0.019
Breast muscle (BMY)	27.55 ± 1.6	28.20 ± 2.1	28.20 ± 1.2	28.13 ± 1.3	0.36
Thigh yield (TY)	42.03 ± 1.2	41.36 ± 2.1	42.53 ± 0.8	42.54 ± 1.1	0.051
Liver index	$2.80^{ab} \pm 0.5$	$3.10^a \pm 0.4$	$2.68^{b} \pm 0.3$	$2.72^{b} \pm 0.4$	0.004
spleen index	0.21 ± 0.04	0.24 ± 0.07	0.24 ± 0.036	0.25 ± 0.4	0.10
Heart index	0.70 ± 0.13	0.71 ± 0.17	0.72 ± 0.10	0.73 ± 0.11	0.864
Bursa index	0.07 ± 0.04	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.04	0.11

^{ab}Means within the same raw with different superscripts are significantly different at P < 0.05; Values are means \pm SD.

Lysoforte Booster® compared to the negative control group. However, breast muscle yield (BMY) and thigh yield (TY) were not affected by the two levels of Lysoforte Booster® supplementation in comparison to negative control group. However, liver indices in the Lysoforte Booster® treatments were significantly (P < 0.01) lower than the positive control group. The improvement observed in some of the carcass traits of birds receiving Lysoforte Booster® may be attributed to the improvement in the overall performance as consequence of proper nutrient utilization efficiency. This confirms the beneficial effect of this absorption enhancer on growth and nutrient utilization. Our results are in agreement with Schwarzer and Adams (1996).

Conclusion

Lysoforte booster is a natural biosurfactant that can significantly improve broiler performance by enhancing feed utilization and nutrient absorption. There are interesting possibilities to improve broiler productivity and to modify carcass quality with Lysoforte and to formulate more cheaper diets with reduced energy and amino acids without any adverse effect on broilers overall performance.

ACKNOWLEDGEMENT

The authors would like to express their appreciation to Kemin Europe, NV.Herentals, Belgium for their valuable financial support to carry out this study.

REFERENCES

Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974). Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

An BK, Nishiyama H, Tanaka K, Ohtani S, Iwata S, Tsutsumi K, Kasa M (1997). Dietary safflower phospholipid reduces liver lipids in laying hens, Poultry Sci., 76: 689–695.

AOAC (1990) Official Methods of Analysis, 15th Edn. Association of Official Analytical Chemists, Washington D. C.

Aoi N (1990). Soy lysolecithin. Yukagaku J., 39(1): 10-15.

Attia YA, Hussein AS, Tag AE, El-Din EM, Qota AI, Abed El-Ghany AM, El-Sudany (2008). Improving productive and reproductive performance of dual-purpose crossbred hens in the tropics by lecithin supplementation. Trop. Anim. Health Prod. (in press) 0049-4747 (Print) 1573-7438 (Online).

Averette LA, See MT, Odle J (2001). Effects of emulsification on amino acid and lipid digestibility in finishing pigs. College of agriculture and life science, Annual Swin report.

Azman MA, Ciftci M (2004). Effects of replacing dietary fat with lecithin on broiler chicken zootechnical performance, Revue de Med. Vet., 1558: 445–448.

Bertoni G, Piccioli Cappelli F, Baldi A, Borghese A, Duranti E, Falasachini A, Formigoni A, Grasso F, Lacetera N, Lupi P, Meluzzi A, Pinna W, Rosi F, Stefanon B, Zicarelli L, Bernabucci U, Campanile G, Moniello G, Trombetta MF (2000). Interpretation of metabolic profiles in farming animals, Progress Nutr., 2: 51–76.

Bovera F, Moniello G, De Riu N, Di Meo C, Pinna W, Nizza A (2007). Effect of diet on the metabolic profile of ostriches (Struthio camelus var. domesticus), Trop. Anim. Health Prod., 37: 265–270.

El-Banna R, Refaie A, Nehad A (2003). Effect of lysine and betaine supplementation on growth performance and breast meat yield of a heavy turkey strain J. Egypt. Vet. Med. Ass., 63(6): 143-157.

Fabiny DL, Ertingshausen G (1971). Automated reaction-rate method for determination of serum creatinine. Clin. Chem., 17: 696-700.

Fossati P, Prencipe L, Berti G (1980). Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzyme assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.

Freeman CP (1984). The digestion, absorption and transport of fat - Non-ruminants, Pages 105-122 in: Fats in Animal Nutrition. J. Wisman, ed. Butterworths, London, England

Gornal AC, Bardawill CJ, David MM (1949). Serum protein determination using colorimetric methods. J. Bio. Chem., 177: 751

Hertrampf JW (2001). Lecithin improves poultry performance, Poult. Int., Nov. 26-30.

Inoue K, Suzuki K, Nojima S (1977). Morphology of Lipid Micefles Containing Lysolecithin J. Biochem., 81(4): 1097-1106

Jones DB, Hancock JD, HarmonD, Walker CE (1992). Effects of exogenous emulsifiers and fat sources on nutrient digestibility, serum lipids, and growth performance in weanling pigs. J. Anim. Sci., 70: 3473–3482.

Khidir MA, Stachecki JJ, Krawetz SA, Armant DR (1995). Rapid inhibition of mRNA synthesis during preimplantation embryo development: vital permeabilization by lysolecithin potentiates the action of &-amanitin. Exp. Cell Res., 219: 619 -625.

Lennox AM, Lough AK, Garton GA (1968). Br. J. Nutr., 22[-237.

Liu D, Veit HP, Wilson JH, Denbow DM (2003). Maternal dietary lipids alter bone chemical composition, mechanical properties, and histological characteristics of progeny of Japanese quail, Poult. Sci., 82: 463–473.

O'Doherty PJ, Kasis G, Kuksis A (1973). Role of luminal lecithin in intestinal fat absorption. Lipids, 8: 249–255.

Polin D (1980). Increased absorption of tallow with lecithin. Poult. Sci.

- 59: 1652.(Abstr.).
- Reitman S, Frankel S (1957). A colorimeteric method for determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. Am. J. Clin. Path., 28: 56-63.
- Reynier MO, Lafont H, Crotte C, Sauve P, Gerolami A (1985). Intestinal cholesterol uptake: comparison between mixed micelles containing lecithin or lysolecithin. Lipids, 20: 145-150.
- Roy AV (1970). Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. Clin. Chem., 16(5): 431-6.
- Schwarzer K, Adams CA (1996). The Influence of specific phospholipids as absorption enhancer in Animal Nutrition. Fett/ Lipid 98 (1996). Nr. 9. S. 304-308. SPSS 2006. SPSS for Windows 14.0.
- Soares M, Lopez-Bote CJ (2002). Effects of dietary lecithin and fat unsaturation on nutrient utilisation in weaned piglets, An. Feed Sci. Tech., 95: 169-177.
- Technical Bulletin Nr.1 (2001). Feed composition tables for animal and poultry feedstuffs used in Égypt. Edited by Central Lab for Food and Feed (CLFF), Ministry of Agriculture, Agricultural Research center, Giza, Egypt.

- UNE (1999). UNE preconex.xls form software linear programming edited by Evan Thompson;
- Wang W, Sunwoo H, Cherian G, Sim JS (2004). Maternal dietary ratio of linoleic acid to α -linolenic acid affects the passive immunity of hatching chicks, Poult. Sci., 83: 2039-2043.
- Wisman J, Salvador F (1989).Influence of age, chemical composition and rate of inclusion on the apparent metabolizable energy value of fats fed to broilers. Poult. Sci., 30: 653-662.
- Xing JJ, van Heugten E, Li DF, Touchette KJ, Coalson JA, Odgaard RL, Odle J (2004): Effects of emulsification, fat encapsulation, and pelleting on weanling pig performance. J. Anim. Sci., 82: 2601-2609
- Zollner N, Kirsch K (1962). Serum total lipids determination colorimetrically. Z. Ges. Exp. Meal., 1335: 545.