

## **LIPID PROFILE OF EDIBLE PARTS FOR BROILERS FED ON THREE REGIMENTS ENRICHED WITH EITHER VEGETABLE OIL OR POULTRY FAT**

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### **ABSTRACT**

Three feeding regiments for broilers were used in the present work, i.e. plant protein diet, (PPD), PPD supplemented with either fish meal (PPDF) or meat and bone meal (PPDM). The Three regiments were enriched with vegetable oil (VO) or poultry fat (PF). Fatty acids content in breast, thigh and skin were determined. Total cholesterol, LDL and HDL cholesterol in blood serum breast and thigh meat was determined. Birds fed on feeds supplemented with PF had higher content of myristic and palmitic acids than those supplemented with VO. Also these birds had the highest content of cholesterol. Cholesterol content of breast and thigh for treatments fed PPD were the lowest compared to those fed on PPDF or PPDM. It is recommended to use vegetable oil in combination with PPD in raising broiler chicks to be sure that poultry meat (breast or thigh) had the lowest cholesterol level and suitable fatty acid profile.

### **INTRODUCTION**

The aim of raising poultry is to produce foods for human beings. In this respect feeding poultry should take into consideration the production of healthy food. The Regional Center for Food and Feed, adapted a feeding system for broilers by using all plant protein diets. This system proved its success in broiler performance and the fine quality of the meat. (Akila *et al* 2004) Diet and its content of fat (fatty acids) and cholesterol are the main reasons for coronary heart disease (CHD) in human beings. Cholesterol is existed essentially from animal protein sources (meats, milk, eggs, fish), while fat existence may, mainly, be provided from plant or animal sources. Cholesterol is a component of cell membranes or a precursor for steroid hormones and bile acids synthesized by cells then absorbed with food. Cholesterol is transported in plasma via High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). LDL cholesterol contributes to atherosclerotic plaque formation within the arterial intima and strongly associated with Coronary Heart Disease (CHD) and related mortality.

Monounsaturated fatty acid enrichment has a positive effect on cardiovascular health, decreasing low-density lipoprotein cholesterol but not high-density lipoprotein cholesterol in blood plasma, and decreasing the susceptibility of low-density lipoprotein to oxidation (Roche, 2001).

There is interest in foods containing higher levels of Polyunsaturated Fatty Acids (PUFA) because of their beneficial effects on human health, mainly in the prevention of cardiovascular disease (Krauss *et al* 2001). For this reason, there are several studies concerning the enrichment of chicken

meat with PUFA by the addition of polyunsaturated fats to the diet (Ajuyah *et al* 1993 and Lopez-Ferrer *et al* 2001).

The present work is an effort to study the effect on the lipoprofile of blood serum and some tissues in broilers fed on either:

- Plant protein diet (PPD).
- PPD supplemented with fish meal (PPDF).
- PPD supplemented with meat and bone meal (PPDM).
- Each diet was enriched with either vegetable oil (VO) or poultry fat (PF).

## **MATERIALS AND METHODS**

The experimental work of the present study was carried out at the Poultry Nutrition Section of the Regional Center for Food and Feed (RCFF), Agricultural Research Center. A total number of 396 unsexed, one-day-old chicks, Cobb broiler strain, were distributed into six equal groups, each of 66 chicks in 6 replicates 11 chicks each.

**The experimental treatments were as follows:**

- The first treatment, group A was fed on a vegetable protein diet (PPD) supplemented with vegetable oil (VO).
- The second treatment, group B was the same as treatment 1 but poultry fat (PF) was used instead of VO.
- The third treatment, group C was fed on fish meal supplemented diet (PPDF) enriched with VO.
- The fourth treatment, group D was the same as treatment 3 but PF was used instead of VO.
- The fifth treatment, group E was fed on meat and bone meal supplemented diet with (PPDF) enriched with VO.
- The sixth treatment, group F was fed as treatment 5 but PF was used instead of VO.

The birds were placed into wire- floored batteries according to the average group weight; the experiment lasted for 38 days. A mash starter, grower and finisher diets contained adequate levels of nutrients for growing broiler diet, producer as recommended by Cobb. The composition of experimental diets are shown in Table (1)

**Measurements:**

**1- Fatty acid content in vegetable oil and poultry fat was determined (Table 2)**

**1-2. Preparation of tissues for fatty acid and total cholesterol determination as follows:**

At 38 days when the average body weight exceeded 1500g marketing size, birds were fasted for 12hrs. The nearest 12 chicks, to the average body weight of their replicate group, were taken and slaughtered. The total number of slaughtered chicks was 72 chicks. Dressed carcass was divided longitudinal in identical halves bone free thigh and breast were taken.

Samples for breast, thigh and skin were separately homogenized and minced before keeping in deep freeze.



### 1-3. Determination of fatty acids in tissues:

Saturated and unsaturated fatty acids were determined in experimental tissues using methyl esters boron trifluoride method (A.O.A.C., 2000). With petroleum ether the fatty substances were extracted from food and feed mixtures after boiling with hydrochloric acid. The extracted oil was saponified with sodium hydroxide in methanol. The fatty acids were methylated with boron trifluoride in methanol, extracted with heptane and determined on a gas chromatography with FID detector (PE Auto system XL) with auto sampler and Ezchrom integration system carrier gas (He); Ca. 25 psi- air 450 ml/ min- Hydrogen 45 ml- split 100 ml / min. oven temperature 200°C injector and detector 250°C

### 1-4. Determination of total cholesterol in tissues.

Total lipid extraction was performed by the procedure described by (Rhee *et al* 1982) the cholesterol content was determined using 3 ml of FeSO<sub>4</sub>- acetic acid and 1 ml of conc. H<sub>2</sub>SO<sub>4</sub> to develop the chromophore. The absorbance was measured at 490nm.

## 2- Blood constituents

### 2-1. Total cholesterol, HDL and LDL

Blood samples were collected randomly from 6 chicks from each treatment. 36 samples were collected at the time of slaughter. Serum was separated by centrifugation at 3000 rpm for 20 minutes and stored at -18 °C until analysis. Total cholesterol was determined according to Naito and Kaplan (1984). HDL and LDL determination was performed according to Rifai *et. al.*, (1999).

**Table (2): percentage fatty acid composition of experimental lipid sources.**

Fatty acid	C. atom	Vegetable oil	Poultry fat
Myristic	C14:0	0.30	0.60
Palmitic	C16:0	6.20	15.70
Stearic	C18:0	3.10	5.50
Oleic	C18:1 $\omega$ 9	26.30	39.30
Linoleic	C18:2 $\omega$ 6	62.30	31.00
Lenolenic	C18:3 $\omega$ 3	0.40	0.70
Vaccenic	C18:1 $\omega$ 7	1.40	7.20
Total sat.*		9.60	21.80
MUFA**		27.70	46.50
PUFA***		62.70	31.70

\*Total sat = total saturated

\*\* MUFA = monounsaturated fatty acid

\*\*\*PUFA = polyunsaturated fatty acid

## RESULTS AND DISCUSSION

Effect of different treatments on some fatty acids concentration in breast, thigh meat and skin

**Palmitic acid:**

Birds fed on PPD, PPDF, PPDM enriched with vegetable oil (VO) (Table 3) gave breast meat fat containing 20.2- 20.3% palmitic acid, being higher than those in thigh and skin, i.e. 18.80-19.90% and 16.10 -17.00% respectively as shown in tables 4 and 5 . No marked effect of the feed was noticed between the three regiments.

Feeding birds on PPD, PPDF and PPDM enriched with poultry fat (PF) instead of (VO) gave higher palmitic acid concentration in tissues under study. Breast meat, gave the highest palmitic acid concentration, being 27.3-28.2 compared to the values of thigh and skin that recorded 26.5-26.9 and 25.4-25.8% respectively.

**Oleic acid:**

The concentration of oleic acid in breast meat, thigh and skin (Tables 3, 4 and 5) were 45.1-45.4%, 41.4-42.1% and 44.1- 44.4% respectively, for birds received feed fortified by VO. However, oleic acid concentration was slightly lower in breast meat, thigh and skin of birds fed on feeds enriched with PF.

**Linoleic acid:**

Treatments receiving VO enriched diets showed the highest linoleic acid concentration, ranging from 21.40- 22.10% in thigh and 14.40-14.50% in breast meat. Skin linoleic acid in this respect ranged 20.60 and 20.80%. Birds grown on diets fortified with PF had the lowest linoleic acid concentration being 12.10-12.30%, 16.00-16.50% and 18.20-18.40% for breast, thigh and skin respectively, (Tables 3, 4 and 5).

**Linolenic acid:**

Linolenic acid concentration was highest in birds fed on VO supplemented diets, being 2.00-2.10%, 0.70- 0.80% and 0.60% for breast, thigh and skin respectively. With birds fed on feeds supplemented with PF, linolenic acid concentration was lower.

**Arachidonic acid:**

Arachidonic acid concentration was highest in tissues for birds fed on VO supplemented feeds, and lowest in those fed on PF fortified feed. No significant difference was noticed due to feed.

**Total saturated fatty acids (SFA):**

Tables 3, 4 and 5 show that total saturated fatty acids was high in treatments supplemented with PF than those enriched with VO. The breast fat showed the highest saturated fats compared to thigh and skin. Differences between the values of thigh and skin were small. No significant differences due to the different regiments (PPD, PPDF, PPDM), were noticed.

**Monounsaturated fatty acids (MUFA):**

The concentration of monounsaturated fatty acid was highest in skin fat followed by breast meat and thigh, for birds fed enriched feeds with VO. Birds fed on feeds fortified with PF gave slightly lower values.

**Polyunsaturated fatty acids (PUFA):**

Polyunsaturated fatty acids in breast, thigh and skin were high in birds fed on feeds supplemented with VO and low in those fortified by PF. No marked effect was noticed as a result of supplementing PPD with either fish meal or meat and bone meal.

**Table (3): Percentage fatty acids concentration in breast meat as affect by different treatments.**

Fatty acid %	Vegetable oil (VO)			Poultry fat (PF)		
	*PPD	**PPDF	***PPDM	PPD	PPDF	PPDM
Myristic C14:0	0.50	0.50	0.50	0.70	0.80	0.80
Palmitic C16:0	20.30	20.30	20.20	28.20	28.00	27.30
Palmitoleic C16:1 $\omega$ 7	6.00	6.70	6.40	7.00	7.00	7.20
Stearic C18:0	7.00	6.00	6.30	8.10	8.00	8.00
Oleic C18:1 $\omega$ 9	45.10	45.10	45.40	39.60	40.00	40.60
Vaccenic C18:1 $\omega$ 7	2.50	2.90	2.80	2.70	2.70	2.60
Linoleic C18:2 $\omega$ 6	14.50	14.40	14.40	12.30	12.30	12.10
Linolenic C18:3 $\omega$ 3	2.00	2.10	2.00	0.30	0.40	0.40
Arachidonic C20:4 $\omega$ 6	2.10	2.00	2.00	1.10	1.00	1.00
Total sat FAs	27.80	26.80	27.00	37.00	36.80	36.10
MUFA	53.60	54.70	54.60	49.30	49.70	50.40
PUFA	18.60	18.50	18.40	13.70	13.70	13.50

\* PPD = Plant Protein Diet

\*\* PPDF = Plant Protein Diet with Fish

\*\*\* PPDM = Plant Protein Diet with Meat and bone meal

**Table (4): Percentage fatty acids concentration in thigh meat as affect by different treatments.**

Fatty acid	Vegetable oil (VO)			Poultry fat (PF)		
	PPD	PPDF	PPDM	PPD	PPDF	PPDM
Myristic C14:0	0.40	0.40	0.40	0.70	0.70	0.60
Palmitic C16:0	18.80	19.90	19.60	26.90	26.50	26.70
Palmitoleic C16:1 $\omega$ 7	6.00	6.00	6.20	6.60	6.70	6.70
Stearic C18:0	5.20	5.30	5.20	6.10	6.60	6.50
Oleic C18:1 $\omega$ 9	42.10	41.40	42.00	39.00	39.40	39.80
Vaccenic C18:1 $\omega$ 7	2.60	2.60	2.60	2.60	2.50	2.00
Linoleic C18:2 $\omega$ 6	22.10	21.70	21.40	16.50	16.00	16.10
Linolenic C18:3 $\omega$ 3	0.80	0.70	0.70	0.60	0.60	0.60
Arachidonic C20:4 $\omega$ 6	2.00	2.00	1.90	1.00	1.00	1.00
Total sat.	24.40	25.60	25.20	33.70	33.80	33.80
MUFA	50.70	50.00	50.80	48.20	48.60	48.50
PUFA	24.90	24.40	24.00	18.10	17.60	17.70

\* See foot not table 2, 3.

**Table (5): Percentage fatty acids concentration in skin for as affect by different treatments.**

Fatty acid	Vegetable Oil(VO)			Poultry Fat(PF)		
	PPD	PPDF	PPDM	PPD	PPDF	PPDM
Myristic C14:0	0.50	0.50	0.50	0.60	0.60	0.60
Palmitic C16:0	16.10	16.70	17.00	25.50	25.80	25.40
Palmitoleic C16:1 $\omega$ 7	6.60	6.20	6.10	5.30	5.10	5.20
Stearic C18:0	6.00	6.20	6.00	6.90	7.00	6.50
Oleic C18: 1 $\omega$ 9	44.40	44.30	44.10	40.30	40.00	40.60
Vaccenic C18:1 $\omega$ 7	4.60	4.50	4.50	2.60	2.50	2.70
Linoleic C18:2 $\omega$ 6	20.80	20.60	20.80	18.20	18.40	18.30
Linolenic C18:3 $\omega$ 3	0.60	0.60	0.60	0.30	0.30	0.40
Arachidonic C20:4 $\omega$ 6	0.40	0.41	0.40	0.30	0.30	0.30
Total sat.	22.60	23.40	23.50	33.00	33.40	32.50
MUFA	55.60	55.00	54.70	48.20	47.60	48.50
PUFA	21.80	21.60	21.80	18.80	19.00	19.00

\* See foot not table 2, 3.

**Cholesterol concentration in blood:**

Table (6) shows the concentration of cholesterol in blood for experimental birds. The lowest values (141.5-142.2 mg/dl) were shown for birds fed diets with VO. In this respect no noticed variability was encountered due to supplementing the PPD with fish meal (PPDF) or meat and bone meal (PPDM). On the other hand, feeds enriched with PF gave higher cholesterol values (153.3-189.1mg/dl). Apparently, with the PF treatments, supplementing the PPD with fish meal or meat and bone meal caused an increase in cholesterol values, which was not the case with VO.

**High Density Lipoprotein (HDL) Cholesterol in Blood:**

HDL values, (Table 6), were the lowest for birds fed on PPD and enriched with VO, being 59.3 mg/dl. This value increased when PPD was supplemented with FM or MBM, reaching values of 62.7 and 71.5 mg/dl respectively. Birds fed on the same feeds but fortified with PF instead of VO showed HDL values ranging between 70.6 and 74.6 mg/dl.

**Table (6): Mean total cholesterol concentration, High Density Lipoprotein cholesterol (HDL) and Low Density Lipoprotein cholesterol (LDL) in bird's blood serum mg/dL.**

Determination	Vegetable Oil (VO)			Poultry Fat (PF)		
	PPD	PPDF	PPDM	PPD	PPDF	PPDM
Total cholesterol	141.5 $\pm$ 2.05	142.2 $\pm$ 1.84	142.1 $\pm$ 0.85	153.3 $\pm$ 1.76	168.3 $\pm$ 2.04	189.1 $\pm$ 1.53
HDL	59.3 $\pm$ 2.69	62.7 $\pm$ 2.66	71.5 $\pm$ 0.68	70.6 $\pm$ 0.93	74.6 $\pm$ 1.87	72.45 $\pm$ 1.82
LDL	33.1 $\pm$ 1.16	32.9 $\pm$ 2.43	28.2 $\pm$ 1.41	34.1 $\pm$ 2.00	33.1 $\pm$ 1.74	34.0 $\pm$ 1.78

\* See foot not table 3.

**Low Density Lipoprotein (LDL) Cholesterol in Blood:**

LDL cholesterol values (Table 6) were lower than HDL values. The differences between treatments were small.

**Cholesterol in breast and thigh tissues:**

From Table (7), it is apparent that breast meat of all treatments contained less cholesterol than that in thigh meat. Diets enriched with VO produced breast and thigh meat with lower cholesterol concentration than those fed on feeds fortified with PF.

PPD diets supplemented with fish meat (FM) or meat and bone meal (MBM) produced breast and thigh meat with higher cholesterol content than the PPD.

**Table (7) Total cholesterol (mg/100g) in breast and thigh tissues of chicken.**

Organs	Experimental treatments					
	Vegetable Oil (VO)			Poultry Fat (PF)		
	PPD	PPDF	PPDM	PPD	PPDF	PPDM
Breast	72.10	76.20	121.05	90.20	95.90	200.90
Thigh	88.05	99.60	151.00	105.60	125.80	231.050

\*See foot not table 3.

It is apparent from the results of the present work, that the concentration of myristic and palmitic acids in poultry fat (Table 2) were higher than those for vegetable oil. Also these two fatty acids were higher in all tissues under study in birds fed the PF supplemented diets (Table 3, 4 and 5). These two fatty acids are hypercholesterolemic, but myristic raises total cholesterol more than palmitic does (**Tholstrup et.al., 1994 and Cater et al., 1997**). This is clearly shown by the cholesterol concentration of breast and thigh in Table 7. It is obvious that birds fed on feeds supplemented with PF had higher cholesterol than those fed on VO.

In conclusion, Table 7 shows that VO treatments gave low breast and thigh meat cholesterol. The use of PF instead of VO increased cholesterol content in both breast and thigh. It is also clear that cholesterol content of breast and thigh of treatments fed on PPD was lowest; either feed was supplemented with VO or PF. In respect to the diets used, the lowest cholesterol content in breast and thigh was 72.1 and 88.05 mg/100g respectively in PPD enriched with VO, followed by 90.20 and 105.60 mg/100g respectively, in PPD enriched with PF.

It may be recommended using vegetable oil with all- plant- protein diets in raising broilers to obtain the poultry meat with lowest cholesterol plus suitable fatty acid profile.

## REFERENCES

- A.O.A.C. (2000). Official Methods of Analysis of the Association of Official Analytical Chemists. 17 Ed. 969.3 and 991.39 Fatty Acids in Oils and Fats Preparation of Methyl Esters Boron Tri Fluoride- AOAC- IUPAC Method Codex-Adopted Method. Chapter 41,p.19-20.
- Ajuyah, A.O., Hardin R.T. and Sim J.S. (1993). Dietary antioxidant and storage affect chemical characteristics of w3 fatty acid enriched broiler chicken meals J. Food Sci. 58: 43-46.
- Akila, S.Hamza; Motawe, H.F.A. and El-Afifi, T.M. (2004). Dietary vegetable protein in broiler feeding. J. Agric Sci. Mansoura univ., 29(7): 3859-3866.
- Cater, N.B.; Heller, H.J. and Denke, M.A. (1997). Comparison of effects of medium chain triacylglycerides palm oil and high oleic sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentration in humans: Am.J.clin. Nutr. 65:48
- Choudhury, N.; Tan, L. and Truswell, A.S. (1995). Comparison of palmolein and olive oil, effect on plasma lipids and vitamin E in young adults. Am.J.clin. Nutr. 61:1045.
- Grande, F.; Anderson, J.T. and keys, A. (1970). Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol. Am.J.clin.Nutr.23:1186
- Krauss, R.M., Eckel, R.H., Howard, B., Appel, L.J., Daniels, S. R., Deckelbaum, R.J., Erdman, J.W., Etherton, P.Kris., Goldberg, I.J., Kotchen, T.A., Lichtenstein, A.H., Mitch, W.E., Mullis, R., Robinson, K., Wylie-Rossett, J., Joer, S.S., Suttis, J., Tribble, D.L. and Bazzare, T.L. (2001). Statement for health- care professional from the nutrition committee of the American Heart Association. J. Nutr. 131: 132-146.
- Lopez- Ferrer, S.; Baucells M.D.; Barroeta A.C. and Grashorn M.A. (2001). N-3 enrichment of chicken meat. 1. use of very long. Chain fatty acids in chicken diets and their influence on meat quality: Fish oil. Poult. Sci. 80:741-752.
- Naito, H.K. and Kaplan, A. (1984). Cholesterol. Clin.Chem. The C.V. Mosby Co. St Louis. Tronto Princeton P.1194- 1206.
- National Research Council (NRC) 1994. Nutrient requirements of poultry 9th Ed; Washington, D.C., USA.
- Rhee, K.S.; Dustson, T.R. and Smilh, G.C.(1982). Effect of changes in intramuscular and subcutaneous fat levels on cholesterol content of raw and cooked beef steaks. J. food Sci. 47.1641.
- Rifai, N.; Bachorik, P.S. and Albers, J.J. (1999). Lipids, Lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors, Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-861.
- Roche, H.M. (2001). Olive oil, high- oleic sunflower oil and CHD. Br.J. Nutr. 85: 3-4.

Tholstrup, T.; Marckmann, P.; Jespersen, J.; Vessby, B.; Jart, A. and Sandstram, B. (1994). Effect on blood lipids, coagulation and fibrinolysis of a fat high in myristic and a fat high in palmitic. Am. J. Clin. Nutr. 60: 922.

### صورة الدهون لمختلف اجزاء الدواجن المغذاة على ثلاث نظم غذائية بإضافة زيت نباتى أو دهن دواجن

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تم استخدام ثلاثة أنظمة غذائية للدواجن : علائق نباتية البروتين، وعلائق نباتية البروتين معززة بمسحوق سمك، وأخرى معززة بمسحوق لحم وعظم. ثم تم إلغاء النظم الغذائية الثلاثة بإضافة زيت نباتى أو دهن دواجن. تم تقدير المحتوى من الأحماض الدهنية فى الصدر و الورك والجلد. كما تم تقدير محتوى الكوليسترول الكلى والكوليسترول منخفض الكثافة والكوليسترول عالى الكثافة فى سيرم الدم و أيضاً تقدير الكوليسترول فى لحم الصدر والورك. ومن التقديرات السابقة وجد أن محتوى حمض الميريستيك وحمض البالمتيك أعلى فى الطيور المغذاة على علف معزز بدهن الدواجن عن الطيور المغذاة على علف معزز بزيت نباتى. أيضاً كانت لحوم هذه الطيور لها أعلى محتوى من الكوليسترول. كما وجد أن محتوى الكوليستيرول فى الصدر والأوراك فى المعاملات المغذاة على علف نباتى البروتين أقل بالمقارنة بالمعاملات المغذاة على علف نباتى البروتين ومعزز بمسحوق سمك أو المعزز بمسحوق لحم وعظم. ولهذا فمن النتائج التى تم الحصول عليها يمكن أن يوصى باستخدام زيت نباتى مع العلف النباتى البروتين أثناء تغذية الدواجن لضمان جودة لحوم الدواجن ( صدر أو ورك) من حيث انخفاض مستوى الكوليستيرول بالإضافة إلى محتوى الأحماض الدهنية.

**Table (1): Percentage for experimental diets and their calculated values.**

Ingredients	Starter						Grower						Finisher					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Meat & bone meal	---	---	---	---	5.000	5.000	---	---	---	---	5.000	5.000	---	---	---	---	5.000	5.000
Fish meal	---	---	5.000	5.000	---	---	---	---	5.000	5.000	---	---	---	---	5.000	5.000	---	---
Vegetable oil	1.609	---	1.004	---	1.726	---	2.299	---	1.735	---	2.205	---	3.967	---	3.435	---	3.901	---
Poultry fat	---	1.799	---	1.193	---	1.915	---	2.569	---	1.925	---	2.545	---	4.510	---	3.895	---	4.421
Y.Corn	58.994	58.804	61.790	61.600	60.410	60.220	63.436	63.166	66.470	66.280	65.000	64.660	63.692	63.149	66.200	65.740	65.065	64.545
Soybean meal 44%	29.200	29.200	25.000	25.000	28.800	28.800	25.000	25.000	21.000	21.000	24.500	24.500	24.260	24.260	20.500	20.500	23.669	23.669
Corn gluten meal	5.860	5.860	3.339	3.339	2.200	2.200	5.000	5.000	2.000	2.000	1.500	1.500	3.746	3.746	1.000	1.000	0.500	0.500
Limestone	1.142	1.142	0.842	0.842	0.336	0.336	1.110	1.110	0.800	0.800	0.300	0.300	1.100	1.100	0.800	0.800	0.290	0.290
Di-Ca-P	1.975	1.975	2.040	2.040	0.399	0.399	1.910	1.910	1.980	1.980	0.340	0.340	1.930	1.930	2.000	2.000	0.360	0.360
C.chloride	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Vit & Min mixture	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Salt	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
DL-Meth	0.087	0.087	0.036	0.036	0.107	0.108	0.100	0.100	0.050	0.050	0.120	0.120	0.120	0.120	0.070	0.070	0.140	0.140
L-Lysine	0.258	0.258	0.074	0.074	0.147	0.147	0.270	0.270	0.090	0.090	0.160	0.160	0.310	0.310	0.120	0.120	0.200	0.200
<b>Total</b>	<b>100</b>																	
<b>Calculated values:</b>																		
ME Kcal/kg	3002	3000	3004	3006	3008	3005	3087	3083	3088	3090	3084	3084	3182	3179	3182	3179	3182	3178
CP%	21.561	21.546	21.548	21.534	21.362	21.307	19.540	19.522	19.340	19.330	19.463	19.440	18.513	18.470	18.526	18.497	18.538	18.505

**A: all plant protein diet + vegetable oil (PPD-VO)**

**C: fishmeal supplement diet + vegetable oil (PPDF-VO)**

**E: meat & bone meal diet + vegetable oil (PPDM-VO)**

**B: all plant protein diet+ poultry fat (PPD-PF)**

**D: fishmeal supplement diet + poultry fat (PPDF-PF)**

**F: meat & bone meal diet + poultry (PPDM-PF)**

\*Vitamin-mineral mixture supplied per kg diet: Vit A=12000 IU; Vit D3= 2000 IU; Vit E=10mg; Vit K3=2mg; Vit B1=1mg; VitB2=5mg; VitB6=1.5mg; Vit B12=10µg; Biotin=50µg; Choline chloride=500mg; Pantothenic acid=10mg; Niacin=30mg; Folic acid=1mg; Manganese=60mg; Zinc=50mg; Iron=30mg; Copper=10mg; Iodine=1mg; Selenium=0.1mg and Cobalt=0.1mg.

