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**EFFECT OF REPLACEMENT OF MAIZE BY ANIMAL FAT ON PLASMA AND MUSCLE
LIPID PROFILE OF LARGE WHITE YORKSHIRE PIGS**

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ABSTRACT

The experiment was conducted to assess the effect of replacement of maize by different levels of animal fat on plasma and muscle lipid profile of pigs. Thirty weaned Large White Yorkshire piglets were randomly divided into three groups and randomly allotted to the three dietary treatments, T1 (control ration as per NRC, 1998), T2 (50 per cent of maize of control ration replaced by animal fat) and T3 (100 per cent of maize of control ration replaced by animal fat). Blood and longissimus dorsi muscle samples were collected for lipid profile estimation. The plasma triglycerides, total cholesterol, HDL, LDL, VLDL cholesterol, LDL:HDL and total cholesterol:HDL ratio of pigs maintained on three dietary treatments ranged from 37.20 to 50.40 mg/dl, 78.60 to 128.60 mg/dl, 37.20 to 52.40 mg/dl, 33.96 to 66.12 mg/dl, 7.44 to 10.08 mg/dl, 1.12 to 1.47 and 2.12 to 2.47, respectively. There was no difference between T2 and T3 groups regarding plasma triglycerides, HDL cholesterol, VLDL cholesterol, LDL/HDL ratio and total cholesterol/HDL ratio. The animals in T1 had lower ($P<0.01$) levels of all plasma lipid parameters observed in the study. There was significant difference ($P<0.01$) among all the three treatments regarding LDL cholesterol levels, the value was higher in 100 per cent replacement group. The muscle triglycerides, total cholesterol, HDL, LDL and VLDL cholesterol of pigs maintained on three dietary treatments ranged from 30.28 to 31.76 mg, 52.29 to 55.11 mg, 25.92 to 26.54 mg, 20.03 to 22.35 mg and 6.05 to 6.35 mg per 100 g and values were statistically similar ($P>0.05$). Though replacement of maize by animal fat elevated the plasma lipid profile of pigs, the muscle lipid profile remains constant. Hence it can be concluded that maize can be replaced by animal fat but more economically at 50 per cent level.

Key words: Maize, Animal fat, Pig, Lipid profile.

INTRODUCTION

Cereal grain forms the major source of energy in the swine feed. Even though, India produces more than 20 million MT of maize per year (Anon., 2012), it could meet only 60 per cent of the requirement in the country. The lower availability and increasing price of maize, necessitates an alternative energy source for incorporation in the swine feed. Animal fat is a byproduct of meat industry and can be included as a source of energy in swine ration. India produces 0.14 million MT of tallow and 0.02 million MT of lard per year (FAO, 2010). The use of fat as an energy source for pigs has been shown to increase digestibility of nutrients, improve growth rate and also reduces dustiness of feeds and, increases palatability. However, studies on the effects of adding animal fat to swine diets have yielded variable results such as increased carcass fat and blood lipid profile.

Cera *et al.* (1989) reported higher for serum triglycerides (60.02 mg/dl) in pigs fed diet with eight per cent of tallow than non supplemented group (32.48 mg/dl).

Jones *et al.* (1992) stated no significant difference in serum total cholesterol, triglycerides, LDL and HDL cholesterol and HDL to LDL ratio between pigs fed diet containing 10 per cent of tallow, lard, soyabean oil or coconut oil. Gallardo *et al.* (2008) reported 77.46, 30.60, 38.19, 43.25 mg/dl for total cholesterol, HDL and LDL cholesterol and triglycerides, respectively in pigs at 45 days of age (fed ration with 18 per cent of CP and 2450 kcal of NE/kg), whereas the corresponding values in pigs at 190 days of age (fed ration with 16 per cent of CP and 2375 kcal of NE/kg) were 125.81, 51.95, 63.42 and 50.04 mg/ dl.

Lack of significant difference in cholesterol content of longissimus muscle of pigs fed different dietary fat sources at four per cent level (tallow, lard, soyabean oil or canola oil) was reported by Fontanillas *et al.* (1997) and Kreuzer *et al.* (2002). Similarly, Grela and Kondek (2000b) and Kouba *et al.* (2003) had reported lack of significance in cholesterol content of loin as a result of supplementing soybean oil and tallow at five per cent. However, Rey *et al.* (2004) reported a mild increase in the

total cholesterol content of longissimus muscle from 33.5 to 37.3 mg/100 g in pigs fed on free range system. Kim *et al.* (2008) recorded 63.63 mg of total cholesterol per 100 g longissimus dorsi muscle of pigs fed commercial diet. Hanczakowski *et al.* (2009) could not find any significant difference in total cholesterol level of longissimus muscle among pigs fed on diet with five per cent each of beef tallow, rapeseed, coconut oil or butter and they concluded that fatty acid profile of pig muscle was stable and was depended to a relatively small extent on the fatty acid composition of dietary fat. Parunovic *et al.* (2012) recorded a total cholesterol concentration of longissimus muscle as 61.7 to 63.1 mg/100 g in pigs fed diet containing maize and soyabean meal.

In the modern world increased consumption of unbalanced rich food has lead to increased incidences of hyperlipidemia, hypercholesterolemia and cardiac problems in human beings. The results obtained in this study can be extrapolated to human beings since their physiological systems are similar. The present experiment was conducted to assess the effect of replacement of maize by different levels of animal fat on plasma and muscle lipid profile of pigs.

MATERIALS AND METHODS

The experiment was conducted to assess the effect of replacement of maize by different levels of animal fat (containing mainly tallow lard and little of poultry fat) on lipid profile in pigs. Thirty weaned female Large White Yorkshire piglets were randomly divided into three groups with five replicates in each group. All piglets

were housed in the same shed and were maintained under identical management conditions throughout the experimental period of 70 days. Restricted feeding was followed by allowing them to consume as much as they could, within a period of one hour and the balance feed was collected and weighed after each feeding. Clean drinking water was provided *ad libitum* in all the pens throughout the experimental period. Daily feed intake was recorded.

The animals were fed with standard grower ration containing 18 per cent of CP and 3265 kcal of ME/kg of feed up to 50 kg body weight and finisher ration with 16 per cent CP and 3265 kcal of ME /kg of feed from 50 kg body weight as per NRC (1998). The three groups of piglets were randomly allotted to the three dietary treatments, T1 (control ration as per NRC, 1998), T2 (50 per cent of maize of control ration replaced by animal fat) and T3 (100 per cent of maize of control ration replaced by animal fat). Ingredient and chemical composition of pig grower and finisher ration were given in the Table 1 and 2. The ration used in this study had similar nutrients as per NRC (1998; 2012) recommendations. Fresh rendered animal fat was obtained from Meat Technology Unit, Department of Livestock products Technology, College of Veterinary and Animal Sciences, Mannuthy as and when the feed was prepared. The animal fat is a mixture of mainly beef fat (tallow) and pig fat (lard) and little of poultry fat. At the end of the experiment five animals from each treatment were slaughtered for muscle and blood sample collection.

Table 1- Ingredient composition of pig grower and finisher rations, %

Ingredients	Grower rations ¹			Finisher rations ¹		
	T1	T2	T3	T1	T2	T3
Yellow maize	70	35	0	74	37	0
Wheat bran	1.5	31	59.8	3.6	34.7	64.9
Soyabean meal	26.25	25.5	25.0	20.5	19.7	19.2
Animal fat	0	6.5	13	0	7	14
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.9	0.4	0	0.65	0.10	0
Calcite	0.85	1.1	1.7	0.75	1.0	1.4
Total	100	100	100	100	100	100
Nicomix AB ₂ D ₃ K ¹ , g	25	25	25	25	25	25
Nicomix BE ² , g	25	25	25	25	25	25
Zinc Oxide ³ , g	45	13	0	30	0	0
Oxylock antioxidant ⁴ , g	10	10	10	10	10	10

¹Nicomix A, B₂, D₃, K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A- 82,500 IU, Vitamin B₂-50 mg, Vitamin D₃-12,000 IU and Vitamin K-10 mg per gram.

²Nicomix BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B₁-4 mg, Vitamin B₆-8 mg, Vitamin B₁₂-40 mg, Niacin-60 mg, Calcium pantothenate- 40 mg and Vitamin E-40 mg per gram.

³Zinc oxide (Nice Chemicals Pvt. Ltd., Kochi) containing 81.38% of Zn.

⁴Oxylock antioxidant (Vetline Ltd., Indore) contains Ethoxyquin, Butylated HydroxyToluene (BHT), Chelators and Surfactant.

Table 2 - Chemical composition* of pig grower and finisher rations

Parameters	Grower rations ¹			Finisher rations ¹		
	T1	T1	T2	T1	T2	T3
Dry matter, %	89.20±0.12	90.56±0.11	91.41±0.13	89.11±0.12	90.41±0.17	91.50±0.18
Crude protein, %	18.25±0.11	18.18±0.17	18.03±0.13	16.39±0.10	16.28±0.06	16.06±0.18
Ether extract, %	3.10±0.05	8.53±0.09	13.69±0.10	3.28±0.06	9.04±0.11	14.11±0.07
Crude fibre, %	3.72±0.11	6.58±0.13	9.42±0.10	3.73±0.07	6.54±0.10	9.40±0.03
Total ash, %	5.64±0.17	9.50±0.20	12.40±0.18	5.54±0.15	9.54±0.12	12.47±0.14
Nitrogen free extract, %	69.29±0.16	57.21±0.21	46.46±0.21	71.06±0.20	58.60±0.30	47.96±0.05
Acid insoluble ash, %	1.10±0.02	4.51±0.09	6.63±0.12	1.04±0.06	4.29±0.13	6.52±0.16
GE, kcal/kg	4132.18 ± 22.92	4134.95 ±14.98	4212.87 ±9.21	4165.18 ±22.24	4203.07 ±17.05	4448.30 ±36.74
Calcium, %	0.59±0.01	0.62±0.006	0.78±0.01	0.62±0.02	0.65±0.01	0.77±0.02
Phosphorus, %	0.58±0.01	0.71±0.01	0.85±0.01	0.55±0.02	0.72±0.02	0.83±0.01
Magnesium, %	0.14±0.006	0.24±0.009	0.40±0.007	0.13±0.008	0.25±0.01	0.37±0.02
Manganese, ppm	16.78±0.38	39.14±1.76	69.99±1.18	16.59±0.45	38.76±0.96	69.85±1.31
Copper, ppm	6.35±0.08	9.34±0.06	12.62±0.19	6.15±0.15	9.17±0.08	12.39±0.15
Zinc, ppm	71.52±1.29	67.19±2.23	88.52±1.15	71.39±1.36	64.95±1.47	88.50±1.62

* On DM basis, ¹ Mean of four values with SE

Table 3 - Lipid profile of plasma and muscle of pigs maintained on the three experimental rations

Lipid profile	Treatments ¹		
	T1	T2	T3
Plasma lipid profile			
**Triglycerides, mg/dl	37.20±1.93 ^a	46.20±1.83 ^b	50.40±4.04 ^b
**Total cholesterol, mg/dl	78.60±3.49 ^a	116.80±3.31 ^b	128.60±4.24 ^b
**High density lipoprotein, mg/dl	37.20±1.91 ^a	49.20±2.40 ^b	52.40±3.11 ^b
**Low density lipoprotein, mg/dl	33.96±2.00 ^a	58.36±1.94 ^b	66.12±1.31 ^c
**Very low density lipoprotein, mg/dl	7.44±0.39 ^a	9.24±0.37 ^b	10.08±0.81 ^b
**LDL/HDL ratio	1.12±0.06 ^a	1.39±0.08 ^b	1.47±0.08 ^b
**Total cholesterol/HDL ratio	2.12±0.06 ^a	2.39±0.08 ^b	2.47±0.08 ^b
Muscle lipid profile*			
Triglycerides, mg/100 g	30.28±0.64	31.48±0.58	31.76±0.77
Total cholesterol, mg/100 g	52.29±1.39	54.57±1.19	55.11±1.18
High density lipoprotein, mg/100 g	26.20±0.37	25.92±0.72	26.54±0.48
Low density lipoprotein, mg/100 g	20.03±1.03	22.35±0.39	22.22±0.64
Very low density lipoprotein, mg/100 g	6.05±0.13	6.30±0.12	6.35±0.15

¹Mean of 5 observations with SE

a, b, c - Means with different superscripts within the same row differ significantly ** (P<0.01)

*Non significant (P>0.05)

PLASMA LIPID PROFILE

Blood samples were collected in clean dry test tube using sodium citrate as anticoagulant on day one of the feeding trial and at time of slaughter. Blood samples were centrifuged at 3000 rpm for 20 min to separate the plasma. Plasma was subjected for lipid profile estimation. Plasma total cholesterol (Lie, 1976), high density lipoprotein cholesterol (HDL) (Haar, 1978) and

triglycerides (Vowan, 1983) were estimated using kit (AGAPPE DIAGNOSTICS LTD., India) by automated blood analyser – Mispa plus. Very low density lipoprotein (VLDL) (triglycerides/5), LDL-low density lipoprotein cholesterol (total cholesterol-HDL-VLDL) and ratios of HDL/LDL, LDL/HDL and total cholesterol/HDL were calculated.

MUSCLE LIPID PROFILE

Longissimus dorsi muscles collected from loin eye area during the slaughter of experimental pigs were subjected to lipid profile study as per Folch *et al.* (1957) with modification. Two grams of muscle sample was homogenized using 20 ml of Folch's solution (chloroform and methanol at 2:1ratio). After one hour of homogenization, filtered through filter paper No.1. Then 5 ml of 0.88 per cent NaCl solution was added and kept for one hour for two layer separation. The top layer was removed carefully and discarded. The bottom layer containing lipid and chloroform was condensed and made up the volume to 1.5 ml. Using this solution lipid profile was estimated using diagnostic kits (AGAPPE DIAGNOSTICS LTD., India).

STATISTICAL ANALYSIS

Data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) method as described by Snedecor and Cochran (1994). Means were compared by Duncan Multiple Range Test (DMRT) using Statistical Package for Social Studies (SPSS. 17.0.1v, 2008) software.

RESULTS AND DISCUSSION

PLASMA LIPID PROFILE

Initial plasma lipid profile of the pigs before starting the experiment were 31.88 mg/dl for triglycerides, 58.13 mg/dl for total cholesterol, 31.13 mg/dl for HDL cholesterol and 20.73 mg/dl for LDL cholesterol. The plasma lipid profile and their ratios in blood collected during slaughter of pigs maintained on four experimental treatments are presented in Table 3.

The three groups on average had 37.20 to 50.40 mg/dl triglycerides, 78.60 to 128.60 mg/dl total cholesterol, 37.20 to 52.40 mg/dl HDL cholesterol, 33.96 to 66.12 mg/dl LDL cholesterol, 7.44 to 10.08 mg/dl VLDL cholesterol, 1.12 to 1.47 LDL: HDL ratio and 2.12 to 2.47 total cholesterol: HDL ratio.

The values recorded in the present study falls within the normal range reported for the species (Sastri,

1985; Cowell, 2004). Control group of pigs recorded lower value for the lipid parameters studied, which is in agreement with Thomas (2007) who reported a value of 57.62 mg/dl for plasma triglycerides, 120 mg/dl for total cholesterol and 43.80 mg/dl for HDL cholesterol in crossbred pigs fed standard ration. Gallardo *et al.* (2008) also recorded similar values for total cholesterol (77.46 to 125.81 mg/dl), HDL cholesterol (30.60 to 51.95 mg/dl), LDL cholesterol (38.19 to 63.42 mg/dl) and triglycerides (43.25 to 50.04 mg/dl) in blood of pigs. Anuraj (2011) reported the values of 83.28 to 101.62 mg/dl for total cholesterol, 26.90 to 47.02 mg/dl for triglycerides and 58.48 to 68.70 mg/dl for HDL cholesterol in Large White Yorkshire pigs fed diet with different levels of dried tuna

waste silage. Sreeparvathy (2011) and Jisha (2012) also reported the similar values in pigs.

From a perusal of the data presented in the Table 3 it can be seen that there was no difference between T2 and T3 groups regarding plasma triglycerides, HDL cholesterol, VLDL cholesterol, LDL/HDL ratio and total cholesterol/HDL ratio. The animals in T1 had lower ($P<0.01$) levels of all plasma lipid parameters observed in the study except for HDL/LDL ratio which was higher. There was significant difference ($P<0.01$) among all the three treatments regarding LDL cholesterol levels, the value increasing with level of animal fat in the diet.

An elevated plasma lipid profile in response to fat supplementation in pigs was reported by Thacker *et al.* (1981) (tallow at 10 per cent), Baldner-Shank *et al.* (1987) (tallow at 15.6 per cent) and Cera *et al.* (1989) (tallow at eight per cent), whereas Jones *et al.* (1992) stated that inclusion of 10 per cent of either tallow or lard in the feed of pigs did not cause any difference in serum total cholesterol, triglycerides, LDL and HDL cholesterol and HDL to LDL ratio.

MUSCLE LIPID PROFILE

The data on lipid profiles of longissimus dorsi muscle such as triglycerides, total cholesterol, HDL, LDL and VLDL cholesterol of pigs maintained on the four experimental rations T1, T2 and T3 are presented in Table 3. The three groups on average had 30.28 to 31.70 mg triglycerides, 52.29 to 55.11 mg total cholesterol, 25.92 to 26.54 mg HDL cholesterol, 20.03 to 22.35 mg LDL cholesterol and 6.05 to 6.35 mg VLDL cholesterol per 100 g of muscle. Statistical analysis of the data revealed no difference ($P>0.05$) between treatments.

In agreement with the results obtained in the present study, no difference in cholesterol content of longissimus dorsi muscle was observed in pigs fed four per cent tallow (Leszczynski *et al.*, 1992), 11 per cent of tallow (Harris *et al.*, 1993), four per cent of tallow or lard (Fontanillas *et al.*, 1997; Kreuzer *et al.*, 2002), five per cent of tallow (Grela and Kondek, 2000; Kouba *et al.*, 2003, Hanczakowski *et al.*, 2009). Even though the level of fat in the feed and type of feed can affect cholesterol level in blood of pigs, it did not affect the muscle lipid profile and the cholesterol content in muscles remained relatively stable (Klingenberg *et al.*, 1995; Martins *et al.*, 2005; Rideout *et al.*, 2008). The result of the present study was in agreement with Rey *et al.* (2004), Kim *et al.* (2008) and Parunovic *et al.* (2012) who reported normal level of total cholesterol concentration in longissimus muscle irrespective of their feeding system or diet fed.

CONCLUSION

Though replacement of maize by animal fat elevated the plasma lipid profile of pigs, the muscle lipid profile remains constant. Hence it can be concluded that maize can be replaced by animal fat but more economically at 50 per cent level.

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