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Research Article

Hypocholesterolemic Effect of Oat Seeds on the Rabbit and Meat Quality

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Authors' Contributions
MHM presemted the idea, conducted the reseach and interpreted the results. MD provide raw material and assisted in research work. MAI helped in animal-keeping and feed preparation. MRT presented the research idea and helped in writing up the manuscript. KA assisted in sampling.

Keywords
Animals feeding, Animal rearing, Rabbit meat, Functional meat

Abstract | Rabbits are reared for meat production in backyard farming system in rural areas of Pakistan. The quality of meat depends upon the nutritious value, texture profile, taste and flavour. Mostly dieticians recommend rabbit meat due to low in caloric value then other traditional meats. To enhance the quality of rabbit meat oat is used as feed for rabbits, because oat is good source of dietary ingredients. In this study 2-4% oat seeds were used to investigate their dietary response. The range of oat that is used in this research will improved the blood lipid and fatty acid profiles and reduced the percentage of fat of rabbit meat. Rabbits were reared for the period of two month in three groups. Slaughtering of rabbits was done after 8 weeks. Followings the method illustrated in previous studies fatty acid and physico-chemical analysis were performed. The pH texture, fat, protein, blood lipid profile and PUFA concentration was significantly affected by the incorporation of oat in rabbit feed. It was concluded that the supplementation of 2% oat in rabbit feed increase n-3 PUFA in meat while improvement in serum lipid profile was observed by 4% supplementation of oat seeds. It was also observed that fat percentage in meat was also reduced by oat seed supplementation.

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Introduction

A food is said to be functional food in which a component has been excluded or already been added by biotechnological or technological ways (Hernández *et al.*, 2008). Functional food provides traditional nutrients along with some specific health benefits like lowering blood cholesterol and source of omega fatty acids. Drugs and supplements show negative effects on health due to that reason functional foods are becoming popular (Drozen and Harrison, 1998). Nowadays everyone needs healthy foods without changing in their dietary style. Re cently, nutritious diet has gained more attention because people dislike carbohydrates, lipids, and salts. People are health conscious (Becker and Kyle, 1998). Consumer have higher demand for healthy diet having low fat or no fat and low in calories (Hilliam, 1995). Functional foods provide an alternative better food to customer and that food should be taken as regular diet (Ohr, 2005). The food is said to be functional if food fulfil three basic requirements. First of all, it is not extracted from naturally occurring ingredient. Secondly, it should be used as daily food and thirdly after consumption of this food, it should treat and prevent from specific disease and regulate specific processes like controlling physical and mental conditions, enhancing biological defense mechanisms and delaying the

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ageing (Goldberg, 1994). In any animal tissues meat is the main part of animal carcass (Williams, 2007). In human nutrition meat plays fundamental role because an ample quantity of minerals, fats, proteins, essential amino acids (EAA) and vitamins are present in meat and meat products (Weiss *et al.,* 2010). Meat is a major source of protein as well as many EAA like histidine, lysine, threonine. Meat also provides biologically active substances, group B vitamins and minerals deposits. Besides all these benefits meats is also linked with hypertension, CVDs (Cardiovascular diseasea), diabetes, and overweight because meat is a source of saturated essential fatty acids and cholesterol (Valsta *et al.,* 2005). Healthy meat products include products with improved fatty acid composition containing omega-3 fatty acid and low level of cholesterol, saturated fats nitrite, nitrate and sodium. The meat processing industry should provide these healthier products by increasing consumer demand (Jiménez-Colmenero *et al.,* 2001). Meat is considered healthy if it is rich in n-3 fatty acids, higher in protein and low in n-6 fatty acids and overall fat. Quality of meat is affected by several interrelating factors like breastfeeding courses, genotype, slaughtering, breed and storing conditions. Among them, quality of meat is reflected by the animal nutrition that plays an important role on biological process in muscle (Andersen *et al.,* 2005). Rabbit meat provides nutritive properties and excellent dietetics. Rabbit lean meat contains high amount of valuable protein (Combes, 2004). Meat of rabbit is low in calories because low amount of saturated fats as compared to red meat. Fatty acid composition of rabbit meat contains high amount of polyunsaturated fatty acids (PUFA) (Dalle Zotte, 2004). Rabbit meat contains 59 mg/100 g cholesterol in muscles tissues that is lesser that other meats (beef 67 mg, chicken 81 mg, pork 59 mg). Addition of oat in rabbit feed enhance the nutritive value of rabbit meat (Combes, 2004). Oat is a good source of water soluble fibre (β-glucan) and these soluble fibre compounds exert beneficial properties on the lipoprotein profile (Kerckhoffs *et al.,* 2003). Oat also provides variety of antioxidants (tocols) and phenolic compounds (Aly, 2012). Antioxidant of oat enhance the scavenging of reactive oxygen species and prevent oxidation of low-density lipoprotein (LDL) (Chen *et al.,* 2006; Stevenson *et al.,* 2008). Past studies demonstrate the ability of oat bran in reducing or enhancing cholesterol and LDL-C concentration or no effect on HDL concentrations in humans (Stevenson *et al.,* 2008; Charlton *et al.,* 2011; Othman *et al.,* 2011). The recent study was conducted to explore beneficial effects of rabbit meat on health. Our objective was to develop a PUFA enriched and low-fat rabbit meat and analyse serum lipid profile.

Materials and Methods

Rabbit's diet and management

The present research was conducted on growing rabbits with oat supplemented feed in different amounts (up to 4%) and evaluate the effect of oat on fatty acids composition and prone to lipids oxidation of the meat. For this study, strains of New Zealand rabbits were obtained from National Institute of Health (NIH) Islamabad. Four hundred and fifty rabbits were reared for 30±5 days and separated into three groups. All these rabbits were reared in animal room in separate cages at 25°C and all cages were fumigated. The diet for rabbits were prepared according to the rabbit's nutritional requirement and the feed was supplemented with oat in two different ratios (2% and 4%) as showed in Table 1. Rabbits were fed ad libitum (according to desire) for two months.

Table 1: Feed Formulation

Ingredients (%)	T0	T1	T2
Fresh Alfalfa	74	71	73
Oat	0	4	2
Calcium-phosphate	1.24	1.24	1.24
Soyabean meal (44 %)	21	20	20
Mineral vitamin premix	1.29	1.29	1.29
Calcium-carbonate	0.68	0.68	0.68
Salt	0.81	0.81	0.81
DL-methionine	0.05	0.05	0.05
Molasses	0.90	0.90	0.90
Total	100	100	100
CP (Crude Protein)	16.43	16.49	16.39
GE (Gross Energy) MJ (Milli joule) /Kg DM (Dry matter)	14.99	14.91	14.87

Slaughtering and sample collection

After 8 weeks, slaughtering of rabbits was done according to rules applied for commercial slaughtering and sample of hind leg and loin meat was done. Methods were approved by the ethical committee of the university. Rabbits were slaughtered by cervical dislocation at farm and for further analysis meat was stored at -4°C in refrigerator (Sanyo, Japan). Samples of Blood (5 ml) were obtained from jugular veins and stored in heparinzed blood sample tubes at -18°C.

Physico-Chemical Analysis of meat

Digital pH meter was used to determine the pH of meat samples. For this purpose 10 g sample was taken and mixed with distil water to get a homogenous mixture then reading was taken by following the official method (Sallama *et al.,* 2004). The water activity, protein, fat and texture of meat was measured by following the respective methods (Cosenza *et al.,* 2004; AOAC, 2000; Carlos *et al.,* 2009).

Fatty acid profile

Fatty acid composition of each sample was through GC (Gas Chromatography) (Aligent Technologies 6890) by running sample in Flame Ionization Detector. In this

Table 2: Physico-Chemical Analysis of loin and hind leg of rabbit meat.

Loin						Hind leg				
Treatment	**pH**	**Aw**	**Protein**	**Fat**	**Texture**	**pH**	**Aw**	**Protein**	**Fat**	**Texture**
T0	5.64± 0.03^{B}	0.842± 0.003^{A}	21.06± 0.05^{C}	1.97± 0.03^{A}	2.40± 0.01^{C}	5.56± 0.02^{B}	0.872± 0.02^{A}	20.88± 0.01^{C}	3.73± 0.04^{A}	2.12± 0.02^{C}
T1	5.70± 0.02^{A}	0.835± 0.003^{A}	22.91± 0.02^{A}	1.63± 0.03^{C}	2.87± 0.02^{A}	5.63± 0.03^{A}	0.865± 0.02^{A}	22.45± 0.01^{A}	3.43± 0.02^{C}	2.63± 0.01^{A}
T2	5.74± 0.03^{A}	0.845± 0.002^{A}	22.05± 0.03^{B}	1.84± 0.02^{B}	2.68± 0.01^{B}	5.65± 0.02^{A}	0.875± 0.01^{A}	21.07± 0.03^{B}	3.58± 0.03^{B}	2.40± 0.02^{B}

Various superscripts specify significant difference between the means ($p \leq 0.05$)

analysis as a carrier nitrogen gas was used at 1.3 ml/min flow rate. According to described method the fatty acid composition was estimated (AOCS, 1998).

Serum bio-chemical Analysis of rabbits

Concentration of triglycerides in blood, total cholesterol, LDL and HDL were estimated by the standard methods (Stockbridge *et al.,* 1989; Assmann, 1979; McNamara *et al.,* 1990; Anoni., 1982).

Statistical analysis

The collected values were analyzed by using the factorial Analysis of Variance (ANOVA) using software (Statistic 8.1). The mean comparison was done by Duncan Multiple Range (DMR) test (Steel *et al.,* 1997).

Results and Discussion

Effect on physico-chemical analysis of loin and hind leg of rabbit meat

The result of physico-chemical analysis of this study was showed in Table 2. Oat Enriched feed of rabbits remarkably affect the pH of both leg and loin meat.Maximum value of pH in T2 is 5.74 and minimum value in T0 is 5.56 in loin meat while values were 5.65 and 5.56 in hind leg respectively. These results illustrated that pH of loin and hind leg of rabbit meat was affected significantly by the oat supplementation in feed. The present study showed that by the supplementation of linseed and oat, pH of hind leg and loin muscles was increased. These results showed similarity with the research of various authors who supplement the rabbit feed with different seed and seed oils that ultimately increased the pH value of rabbit meat (Eiben *et al.,* 2010). There is no significant effect of water activities on both loin and leg meats 0.842 and 0.872 respectively. The water activity range of summarized results is not suitable for the growth of different microbes as illustrated by researchers in various foods for various microbes (Pla *et al.,* 2004). Protein percentage of both loin and hind leg meat was significantly affected by oat supplemented feed. In loin meat control rabbit (T0) displayed lowest percentage i.e. 21.06 and T1 presented highest percentage i.e. 22.91 while in case of hind legs values were 20.88 and 22.45 correspondingly. The outcomes of findings related to loin meat exhibited higher protein percentage as compared to rabbit's hind leg meat. This result showed similarity with the past findings (Pla, 2008). Oat supplementation increase biological protein in the feed so overall protein contents increased as compared to control. Fat contents of hind leg and loin meat is also affected by the supplementation of oat in feed. In case of loin meat maximum value for fat percentage is showed in T0 i.e. 1.97 and T1 showed minimum value i.e. 1.63 whereas values were 3.73 and 3.43 in case of hind leg meat for T0 and T1 correspondingly. Oat supplementation in rabbit's diet balanced in protein and digestible energy but did not altered weight of carcass and fatness, as estimated by the perennial and scapular fat percentage. The result of this research is correlated with the finding of various scientist that loin meat has less fat percentage regarding rabbit's hind leg meat (Pla *et al.,* 2004). Oat enrichment in diet significantly effected textural attributes of rabbit meat. In loin meat T0 displayed lowest value i.e. 2.40 and T1 presented highest value i.e. 2.87 while in hind leg meat the values are 2.12 and 2.63 respectively. The summarized results are correlated with the findings of other researchers who illustrated that texture share values decrease as the fat content of meat is increased (Pla, 2008). More fat contents and less texture values of hind leg portion as compared to loin meat of rabbit. The result showed that rabbit meat enriched with long chain PUFA through diet significantly affect the instrumental texture properties. The observed results showed similarity with the findings of author who illustrated that rabbit meat showed lower textural values when feed was enriched with dietary n-6 and n-3 fatty acids (Bianchi *et al.,* 2006).

Fatty acid composition

Most important quality attributes of fats and oils is determined by chain length of fatty acid and degree of unsaturation. Fatty acid composition of rabbit loin meat in showed in Table 3. Addition of dietary oat in the di*et al*tered the fatty acid composition. Table 3 illustrated that the oat seeds resolute contents of saturated fatty acids are less and PUFA are more in both meats (loin and leg). T0 presented highest value i.e. 33.98 and 35.98 in hind leg and loin meat respectively while T1 showed minimum val -ue i.e. 32.44 and 34.01 in hind leg and loin meat respecti-

Table 3: Fatty acid profile (% of total fatty acid)

Fatty acid profile of loin meat				Fatty acid profile of leg meat			
Fatty acid composition (%)	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**p-value**
C14,0	1.84	1.21	1.64	0.94	1.25	0.93	*
C16,0	26.79	25.83	26.28	26.21	25.91	26.03	*
C18,0	7.32	6.97	6.24	6.83	5.28	5.68	Ns
SFA	35.95	34.01	34.16	33.98	32.44	32.64	**
C14,1	0.07	0.02	0.02	0.13	0.07	0.08	Ns
C16,1	0.61	0.95	1.01	1.77	2.27	2.33	**
C18,1	23.03	23.75	23.93	24.57	25.13	25.17	*
MUFA	23.71	24.72	24.96	26.47	27.47	27.58	**
C18,2	18.82	18.04	18.2	20.02	19.65	19.45	*
C18,3	2.98	4.04	4.19	5.1	5.95	6.76	**
C20,3	0.30	1.75	1.81	0.33	1.8	1.66	**
C22,5	0.04	0.03	0.09	0.04	0.04	0.01	*
C22,6	0.09	0.04	0.61	0.25	0.08	0.06	Ns
PUFA	22.23	23.9	24.9	25.74	27.52	27.94	*
UFA	45.94	48.62	49.86	52.21	54.99	55.52	*
SFA/UFA	0.78	0.70	0.69	0.65	0.59	0.59	Ns
PUFA/SFA	0.62	0.70	0.73	0.76	0.85	0.86	*

*significant ** highly significant Ns Non-significant

vely. The concentration of MUFA increased by oat enrichment. T2 showed maximum value i.e. 27.58 and 24.96 in hind leg and loin meat respectively. Growing level of omega PUFA ($P < 0.0001$) was also observed from control group (2.98 % in loin meat, 5.10 % in hind leg meat) toward T2 with 2% oat (4.19 % in loin, 6.67% in hind leg meat) T1 with 4% oat (in loin 4.04%, in hind leg meat 5.95%). Due to highest concentration of ALA (Alpha Linolenic acid) concentration of n-3 PUFA is also higher that define the key fatty acids of oat (Lopez-Bote., 1998). Composition of Fatty acid of Rabbit's meat is also improved by oat containing feed. The observed results are similar with the findings of researchers who supplement feed with 30% oat and found good PUFA/SFA ratio (Stanley *et al.*, 2007). Higher amount of omega 3 enable rabbit meat to reduce overall n-6/n-3 polyunsaturated fatty acid as showed in Table 3. Concentration of EPS (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) was not too higher (approximately 0.10%) and it was not increased from control T0 to T2. Further research needed to estimate the whole oat efficiency to increase the concentration PUFA and α-linolenic acid in rabbit meat and other species (Bernardini *et al.*, 1999; Maertens *et al.*, 2008).

Supplemented feed with 2% dietary oat seeds determined 8.13 % contents of n-3 PUFA of total fatty acids in hind leg. It was concluded that 96mg n-3 PUFA/100g of meat that showed 19% of RDA (Recommended Dietary Allowance) for omega 3 PUFA (EFSA, 2009).

Serum bio-chemical profile

Supplemented rabbits feed with oat significantly affect the blood triglycerides, high density lipoprotein (HDL), low density lipoprotein (LPL) and serum cholesterol concentration. The result summarised in Table 4 regarding effect of oat supplemented feed on blood triglycerides, LDL, HDL and serum cholesterol concentration of rabbit meat. The highest values observed in T0 i.e. triglycerides (85.33mg/dl), serum cholesterol (142.33 mg/dl) and LDL (35.33 mg/dl) the lowest value observed in T1 (4% oat) i.e. triglycerides (66.67 mg/dl), serum cholesterol (105.27 mg/dl) and LDL (27.29 mg/dl). T1 showed highest HDL concentration i.e. 47.67 mg/dl. It is concluded from the result that the concentration of total triglycerides, serum cholesterol and LDL reduce but HDL concentration increase significantly with oat supplemented feed as compared to rabbits without oat supplemented feed. Various studies indicate that major active cholesterol reducing agents in oat is beta-glucan. The reduction of blood cholesterol is significantly greater as the beta-glucan level increase in feed in dependent manner (Behall *et al.*, 1997). Viscous gel formation due to soluble fibre in oat that reduce blood cholesterol. Soluble fibre also binds bile salts and increase their excretion from body (Judd and Trusswell, 1981; Marlett *et al.*, 1994). An independent risk for atherosclerosis is due to high serum triglycerides concentration because it is also a big cause of coronary heart diseases. The results of present study showed that with the supplementation of feed with 4% oat reduced the triglycerides level. The result of current study showed similarity

with the results of different researches who persistent the efficiency of oat in changing blood lipid profile and exert a negative histological effect on hypercholesterolemic male rats (El Rabey *et al.*, 2013). Oat supplemented rabbits feed reduced total triglycerides as compared to control group. In different studies, oat supplemented feed improves concentration of HDL cholesterol in blood significantly and apolipoprotein A-I, an important component of HDL (Turnbull and Reeds, 1989). The results of this study showed HDL contents of rabbits increase significantly with the supplementation feed with 4% oat.

Table 4: Effect of oat supplementation on total serum cholesterol (mg/dl)of rabbits

Treatments	Cholesterol	Triglycerides	HDL	LDL
T0	142.33	85.33	44.33	35.33
T1	105.27	66.67	47.67	27.29
T2	117.78	71.37	41.00	31.52

Changing in the lipid profile, higher ration of HDL to LDL that increase lipoprotein lipase activity that also increase the triglycerides circulation as fuel and enhance their clearance. Activation of lipoprotein lipase speed up the formation of HDL from very low-density lipoproteins (VLDL) (Press *et al.*, 2003). Plasma levels of TC (Total cholesterol) and LDL-C (Low density lipoprotein cholesterol) is potentially effective due to soluble fibre present in oat. In past studies it was reported that soluble fibres reduced lipids both in human and animals (Ruberfroid, 1993; Sawashita *et al.*, 2006).

Conclusions and Recommendations

In this study, results showed that rabbit meat increased in n-3 PUFA when growing on oat supplemented feed. Further it was also concluded that seed supplementation with 2% oat reduced percentage of fat in rabbit's meat. Supplementation of oat up to 4% in diet can also improve the serum lipid profile. Further clinical studies required to prove the ability of rabbit meat containing PUFA in reducing level of cholesterol in human subjects

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