

EFFECTS OF TOTAL MIXED RATION WITH DIFFERENT LEVELS OF *Lupinus angustifolius* L. ON GROWTH PERFORMANCE AND FATTY ACIDS PROFILE OF LONGISSIMUS DORSI MUSCLE OF MALE BOER GOATS

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ABSTRACT. This study aimed to investigate the effects of feeding total mixed ration (TMR) with different levels of lupins grain (*Lupinus angustifolius*) on growth performance and fatty acids of the longissimus dorsi muscle. Boer goats aged 8 to 9 months were divided into 3 groups (n=8) and fed a trial for 103 days on total mixed ration (TMR) diets containing different levels of lupin: CON (0% lupin), TMR A (10% lupin) and TMR B (30% lupin). The final weight, average daily gain (ADG), total weight gains and feed conversion ratio (FCR) for all the experimental groups were similar ($p>0.05$). However, there was a significant decrease ($p<0.05$) in the total feed intake (TFI) and dry matter intake (DMI) for the TMR B group compared to the CON and A groups. The experimental diets altered the proportions of some of the fatty acids in the meat, however the total SFA, MUFA and PUFA as well as the n-6:n-3 and PUFA:SFA ratios were unaffected. The study results indicate the potential utilisation of TMR feed with different levels of lupin in Boer goat feeding.

Keywords: lupin (*Lupinus angustifolius*), Boer goat, growth performance, fatty acid, longissimus dorsi

INTRODUCTION

Goat farming in Malaysia is striving for its competitiveness and sustainable solutions

to reduce feed cost and to enhance growth in consumer demand for locally produced goat products such as chevon. There are several constrains and factors that can affect small ruminant production in Malaysia such as meat preferences of the consumer (Kaur, 2010), government policies, land availability, feed cost and efficiency, suitability of breeds and production system, skilled labour and technical support together with extension services (Shahudin *et al.*, 2018; Devendra and Liang, 2012). Research to increase efficiency in ruminant feeding is critical because of limitations in terms of quality and variability in nutritional value of local feed resources.

With the good nutritional values particularly in crude protein content (30% to 42%), lupins have been reported to give comparable results when used as a replacement for soya bean meal (SBM) in livestock feed (Glencross, 2001; Lee *et al.*, 2016; Lestingi *et al.*, 2016). Lupin is not suitable to be commercially planted in Malaysia due to its high tropical temperature, rainfall pattern and monsoon season that will hamper the production cycle of lupin in its required agroecology. The usage of lupin in Malaysian goat feed is still very

low since it is seasonally imported from Australia particularly when the price is comparable with soybean price. In Malaysia, despite chevon being commonly preferred by certain ethnic groups, the consumption was still low due to the misunderstanding that chevon is unhealthy due to its high cholesterol and saturated fatty acids (Kaur, 2010). Meat is one of the major sources of fats in human diets, especially saturated fatty acids (SFA), which have negative implications on health (Wood *et al.*, 2004). The fatty acid composition of meat is affected by several factors including species, breed, anatomical location, diet and feeding regime (Pitchford *et al.*, 2002; Wang *et al.*, 2003). In addition, fatty acid composition of the meat also plays a crucial role in influencing several aspects of the meat quality, particularly flavour, tenderness, and shelf life (Wood *et al.*, 2004). Thus, fatty acid composition is also an important factor in determining the nutritional and organoleptic quality of the meat. Creating consumer awareness on the right terminology (chevon for goat meat; lamb and mutton for sheep meat), differences of nutritional and meat quality between goat meat or chevon and sheep meat, lamb or mutton is very important in developing better growth of chevon marketing in Malaysia. Thus, the objective of this research was to determine the effects of TMR feeding with different levels of lupin on the growth performance and fatty acid profiles of longissimus dorsi muscle (LD) of male Boer goats.

MATERIALS AND METHOD

Experimental Animal Management and Feeding

The feeding trial experiment was performed in a Department of Veterinary Services goat farm – Pusat Pembiakan Kambing, Kampung Kuala Pah, Kuala Klawang, Negeri Sembilan. The experiment was conducted according to the animal ethic guideline approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia. Twenty-four Boer goats, ages 8 to 9 months old with average mean initial body weight of 27.83 kg were allotted randomly into three treatment groups of eight animals each. Animals were treated for endo and ecto-parasites, provided with anti-stress in drinking water *ad libitum*, adapted to the experimental pen environment and fed for 14 days before the feeding trial. During the feeding trial that lasted for 103 days, animals were fed twice at 09:30 and 14:30 daily *ad libitum* and given free access to clean drinking water. Each animal was kept in an individual pen located in a well-ventilated barn with plastic coated expanded metal floor 1.04 m × 1.40 m (i.e. 1.45 square meters per animal). The total mixed ration (TMR) feeds (Table 1) used in the feeding trial were divided into 3 treatment groups which contained either 13% of soya bean meal, namely the CON (no Lupin group), TMR A (10% lupin) or TMR B (30% lupin). The loose form of TMR diets were adjusted to be isocaloric (metabolisable energy about 10.3 MJ/kg) and isonitrogenous (crude protein about 16.3%) using the mixture of *Brachiara humidicola* hay and concentrate ingredients which

includes palm kernel expeller (PKE), ground corn, wheat pollard, broken rice, palm oil, molasses, salt and vitamin and mineral premix. Lupin grain (*Lupinus angustifolius*), imported from Australia, was ground using a hammer mill through a 3 mm screen before being mixed into the TMR. Body weights were determined on a weekly basis and the growth performance assessed by the weights and average daily gain (ADG). Individual feed intake was measured by recording daily feed offered and refusals. Feed samples were collected biweekly and combined as composite samples for chemical analysis as well as fatty acids composition. Proximate analysis was conducted to determine the chemical composition of the experimental diets according to the methods outlined by AOAC (AOAC, 2007). Samples of the experimental diets were collected from each mixed diet during the

feeding trial. The feed was analysed for dry matter (DM), ash, crude protein (CP), ether extract (EE), calcium, phosphorus, energy content, neutral detergent fibre (NDF) and acid detergent fibre (ADF). The chemical composition and the fatty acid profile of the dietary treatments are presented in Tables 1 and 2 respectively.

Growth Performance Evaluation

Throughout the feeding trial, the live weight of each animal was taken every week, and average daily weight gained was calculated. The amounts of feeds offered and refused by the animals were recorded daily. Daily feed intake of animals were calculated. Feed conversion ratio (FCR) was determined by dividing the amount of feed intake (kg/DM) to body weight gained (kg) of animals at the end of the trial.

Table 1. Proximate analysis of the experimental diets.

Chemical composition (% DM)	CON (n=8) 0% lupin	TMR A (n=8) 10% lupin	TMR B (n=8) 30% lupin	p-value
DM	91.90 ± 0.19	92.63 ± 0.20	92.75 ± 0.36	0.07
CP	17.40 ± 0.34	17.03 ± 0.18	16.75 ± 0.17	0.19
CF	17.51 ± 0.83	17.59 ± 0.55	18.79 ± 0.65	0.36
ADF	25.36 ± 0.61 ^{ab}	26.87 ± 0.53 ^b	24.47 ± 0.50 ^a	0.02
NDF	47.44 ± 1.60 ^b	51.14 ± 1.52 ^b	42.77 ± 0.87 ^a	0.00
EE	3.94 ± 0.11 ^a	4.55 ± 0.13 ^b	5.40 ± 0.09 ^c	0.00
ME i.e. (MJ/kg) ²	10.30 ± 0.17	10.31 ± 0.13	10.51 ± 0.14	0.53
Ca	0.40 ± 0.04 ^b	0.41 ± 0.03 ^b	0.25 ± 0.02 ^a	0.01
P	0.46 ± 0.05	0.50 ± 0.06	0.34 ± 0.03	0.05
Ash	6.89 ± 0.14 ^b	7.01 ± 0.05 ^b	5.65 ± 0.12 ^a	<0.0001

Mean ± S.E.M: superscripts a, b and c mean within a row with no common superscripts differ significantly at $p < 0.05$.

CON: control (basal diet); TMR A: Total Mixed Ration A; TMR B: Total Mixed Ration B; DM: dry matter; CP: crude protein; CF: crude fibre; ADF: acid detergent fibre; NDF: neutral detergent fibre; EE: ether extract; ME: metabolisable energy; Ca: calcium; P: phosphorus.

Table 2. Fatty acid composition of experimental diets.

Fatty acid composition	CON	TMR A	TMR B	p-value
C14:0	7.52 ± 0.31	5.08 ± 1.54	5.11 ± 1.51	0.41
C15:0	0.25 ± 0.14	0.33 ± 0.03	0.35 ± 0.02	0.69
C16:0	19.82 ± 0.39	21.33 ± 2.04	21.88 ± 2.22	0.72
C18:0	3.55 ± 0.15	3.48 ± 0.46	3.84 ± 0.33	0.79
C18:1n-9	24.56 ± 0.16	29.62 ± 3.88	29.61 ± 3.48	0.48
C18:2n-6	20.64 ± 0.57	23.53 ± 0.85	23.08 ± 0.03	0.08
C18:3n-3	1.57 ± 0.15	1.65 ± 0.12	1.51 ± 0.10	0.77
Total SFA	39.97 ± 0.62	35.91 ± 1.34	36.56 ± 0.70	0.10
Total MUFA	31.53 ± 0.06	34.55 ± 2.20	34.56 ± 2.28	0.49
Total n-3 PUFA	2.02 ± 0.18	1.93 ± 0.01	1.78 ± 0.21	0.63
Total n-6 PUFA	26.48 ± 0.50	27.62 ± 0.87	27.10 ± 1.37	0.74
Total UFA	60.03 ± 0.62	64.09 ± 1.34	63.44 ± 0.70	0.10
n-6: n-3 ratio	13.22 ± 0.92	14.32 ± 0.53	15.31 ± 1.03	0.35
UFA: SFA ratio	1.50 ± 0.04	1.79 ± 0.10	1.74 ± 0.05	0.12
PUFA: SFA ratio	0.71 ± 0.03	0.82 ± 0.01	0.79 ± 0.03	0.09

The data are expressed as a normalised percentage (%) of fatty acids. Mean ± S.E.M. CON: control (basal diet); TMR A: Total Mixed Ration A; TMR B: Total Mixed Ration B; SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid; UFA: unsaturated fatty acid; Total SFA = C14:0 + C15:0 + C16:0 + C18:0; Total MUFA = C18:1n-9; Total n-3 PUFA = C18:3n-3; Total n-6 PUFA = C18:2n-6; Total UFA = Total MUFA + Total n-3 PUFA + Total n-6 PUFA; n-6: n-3 ratio = Total n-6 PUFA : Total n-3 PUFA; UFA: SFA ratio = Total UFA : Total SFA; PUFA: SFA ratio = (Total n-3 PUFA + Total n-6 PUFA) : Total SFA

Slaughtering And Muscle Sampling

At the end of the 103-day feeding trial, all animals were transferred to Senawang Abattoir Complex of the Department of Veterinary Services in the evening (19:00 to 20:00) using a lorry. The animals were subjected to overnight lairage to reduce stress and glycogen depletion. During lairage, only *ad libitum* amounts of drinking water was provided to the animals. The weight of each animal was taken before the slaughtering and recorded as empty

live weight. All animals were slaughtered according to the standard procedure (DSM, 2009).

Following exsanguination, the head was removed at the atlanto-occipital joint from the carcass. The right half was dissected immediately for muscle sampling. LD were collected for fatty acid composition evaluation. These muscle tissues were wrapped in aluminium foil, placed in polyvinyl chloride plastic bags and stored at -80 °C until further analysis.

Fatty Acid Composition

Fatty acid composition of the experimental diets and of LD were determined (Ebrahimi *et al.*, 2014).

Experimental diets were ground into a powder form using a grinder (IKA Analysen technik GmbH, Germany) while the tissues were thawed and cut into small pieces.

Total Lipid Extraction

Total lipid was extracted from 0.5 g of experimental diets and 1.0 g of muscle tissues. The weighed samples were mixed with 10 ml chloroform:methanol (2:1, v/v) and 5 ml of normal saline solution. The mixture was mixed vigorously with a vortex mixer for 1 min before centrifugation at $3,000 \times g$ at room temperature for 5 min. After centrifuging, water content at the upper phase was removed while the lipid content at the lower phase was transferred to a cleaned cap methylation tube.

Fatty Acid Methyl Ester Preparation

The methylation of extracted total lipids to fatty acid methyl ester (FAME) was conducted (AOAC, 2007) using 14% methanolic boron trifluoride (BF₃) (Sigma Chemical Co. St. Louis, Missouri, USA).

One hundred microliter of henicanoic acid (C21:0) with a known concentration was added to each sample before methylation as an internal standard to individually quantify fatty acids within the sample. Prior to methylation, the extracted lipid was air-dried on a water bath (60 °C) under a mid-stream of pure nitrogen gas

(99.9%, MOX Sdn Bhd, Malaysia). About 2 ml of 0.66 N methanolic potassium hydroxide was added to the tube to saponify the lipid. The methylation tube was then gassed with nitrogen and heated in a boiling-water bath for 10 min with occasional shaking. After that, 2 ml of 14% methanolic boron trifluoride was added to initiate esterification, and the mixture was reheated for 20 min in the boiling-water bath with occasional shaking. After cooling to room temperature, 4 ml of distilled water and 4 ml of petroleum ether (boiling point 40 °C to 60 °C) were added to the mixture. The mixture was vortexed for 1 min and then centrifuged at $1500 \times g$ for 10 min to facilitate phase separation. The upper phase containing FAME was then transferred to a 4 ml screw-capped vial and stored at -20 °C for analysis by gas-chromatography.

Fatty Acid Methyl Ester Quantification

The FAME were quantified by gas chromatography (Agilent 7890N) using a 30 m \times 0.25 mm internal diameter (0.20 μ m film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). The injector temperature was programmed at 250 °C and the detector temperature was 300 °C. The column temperature program initiated runs at 100 °C for 2 min and was warmed to 170 °C at 10 °C/min. After that, it was held for 2 min, warmed to 200 °C at 7.5 °C/min, and then held for 20 min to facilitate optimal separation.

Determination of fatty acids was conducted by comparing relative FAME peak retention times of samples to standards (Sigma, St. Louis, Missouri, USA). To figure out the total lipids and differences in

fatty acid composition, both gravimetric calculations and normalised percentages of total fatty acids were used. Using a personal computer integrator (Hewlett-Packard, Avondale, PA), peak determination and calibration was calculated. To obtain automatic expression of the peak areas as total and percentage amount of a detected fatty acid, a programmed personal computer using MS Excel 2000 (Microsoft Corporation, Redmond, USA) was used.

Data Analysis

All data were analysed using SAS software (version 9.1, SAS Inst. Inc., Cary, North Carolina, USA) and statistically tested at 95% confidence level. The effects of experimental diets on the growth performance and fatty acid composition of muscles were analysed using the one-way analysis of variance (ANOVA) procedure of a general linear model (PROC GLM). The statistical model used is $Y_i = \mu + \beta_i + \epsilon_i$, where μ is the overall mean, β_i the effect of the different treatment and ϵ_i the residual error. Significantly different

mean values were further analysed using Duncan's test.

RESULTS AND DISCUSSION

Growth Performances

The growth performances (Table 3) show that there were no significant differences ($p > 0.05$) in the final weight, ADG, total weight gain and FCR for all experimental groups. However, total feed intake (TFI) for TMR B group (110.76 ± 3.57) was significantly lower ($p < 0.05$) than CON (135.05 ± 5.04) and TMR A (125.07 ± 3.60).

Lower TFI in TMR B observed in this study may be due to the presence of alkaloids in lupin that reduced the intake or even make seeds less palatable to the animals (Hawthorne, 2006). Despite lower TFI in TMR B, FCR of the goats were similar among the experimental diets in the present study, which is in accordance with Moss *et al.* (Moss *et al.*, 1997).

Hence, the TMR feed with inclusion of lupin as a dietary protein source in the

Table 3. Feed intake and growth performances of Boer goats fed on different diets.

Item	Dietary treatments			p-value
	CON	TMR A	TMR B	
Initial Weight (kg) ^{ns}	28.31 ± 1.02	27.19 ± 0.75	28.00 ± 0.98	0.68
Final Weight (kg) ^{ns}	47.19 ± 1.45	44.31 ± 1.13	43.69 ± 0.80	0.10
Weight Gain (kg) ^{ns}	18.88 ± 1.03	17.13 ± 0.91	15.69 ± 0.88	0.08
Average Daily Gain (kg) ^{ns}	183.25 ± 9.95	166.26 ± 8.79	152.31 ± 8.55	0.80
Total Feed Intake (DM kg)*	135.05 ± 5.04 ^b	125.07 ± 3.60 ^b	110.76 ± 3.57 ^a	0.00
Feed Conversion Ratio ^{ns}	6.51 ± 0.26	6.65 ± 0.24	6.45 ± 0.29	0.86

Mean ± S.E.; ns = non-significant; * = a, b values different superscript different at $p < 0.05$

Table 4. Effects of TMR with different levels of lupin on fatty acid composition of longissimus dorsi muscle in Boer goats.

Fatty Acid Composition	CON (n = 8)	TMR A (n = 8)	TMR B (n = 8)	p-value
C12:0	0.65 ± 0.08 ^b	0.56 ± 0.07 ^b	0.94 ± 0.12 ^a	0.02
C14:0	2.97 ± 0.28	3.03 ± 0.36	2.50 ± 0.34	0.47
C14:1	0.57 ± 0.11	0.59 ± 0.08	0.54 ± 0.07	0.93
C15:0	0.48 ± 0.07	0.65 ± 0.08	0.66 ± 0.06	0.16
C15:1	1.83 ± 0.25	1.90 ± 0.20	1.85 ± 0.20	0.98
C16:0	20.57 ± 0.72	20.50 ± 0.64	20.76 ± 0.86	0.97
C16:1	3.77 ± 0.29 ^a	3.15 ± 0.10 ^b	3.07 ± 0.14 ^b	0.04
C17:0	0.42 ± 0.04	0.53 ± 0.06	0.58 ± 0.09	0.27
C17:1	0.53 ± 0.03	0.59 ± 0.09	0.64 ± 0.05	0.47
C18:0	11.58 ± 0.56	11.75 ± 0.40	12.62 ± 0.67	0.38
C18:1	44.22 ± 1.12	44.34 ± 1.05	44.20 ± 1.06	0.99
C18:2n-6	6.55 ± 1.15	5.74 ± 0.40	5.08 ± 0.30	0.37
C18:3n-3	0.42 ± 0.07 ^b	0.57 ± 0.11 ^{ba}	0.76 ± 0.09 ^a	0.04
C20:4n-6	4.09 ± 0.65	4.58 ± 0.45	4.40 ± 0.44	0.80
C20:5n-3	0.41 ± 0.04	0.58 ± 0.10	0.44 ± 0.05	0.20
C22:5n-3	0.44 ± 0.05	0.45 ± 0.05	0.52 ± 0.08	0.60
C22:6n-3	0.51 ± 0.10	0.49 ± 0.10	0.43 ± 0.09	0.82
Total SFA	36.66 ± 1.16	37.02 ± 1.31	38.07 ± 1.63	0.76
Total MUFA	50.93 ± 0.96	50.57 ± 1.06	50.31 ± 1.11	0.92
Total n-3 PUFA	1.78 ± 0.18	2.09 ± 0.17	2.15 ± 0.16	0.28
Total n-6 PUFA	10.64 ± 1.31	10.32 ± 0.81	9.47 ± 0.72	0.69
Total UFA	63.34 ± 1.16	62.98 ± 1.31	61.93 ± 1.63	0.76
n-6: n-3 ratio	6.07 ± 0.70	5.18 ± 0.57	4.51 ± 0.39	0.18
UFA: SFA ratio	1.75 ± 0.08	1.72 ± 0.10	1.66 ± 0.10	0.78
PUFA: SFA ratio	0.35 ± 0.05	0.34 ± 0.03	0.31 ± 0.03	0.81

The data are expressed as a normalised percentage (%) of fatty acids. Mean ± S.E.M. Superscript a, b mean within a row with no common superscripts differ significantly at $p < 0.05$. CON: control (basal diet); TMR A: Total Mixed Ration A; TMR B: Total Mixed Ration B; SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid; UFA: unsaturated fatty acid. Total SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0; Total MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1; Total n-3 PUFA = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; Total n-6 PUFA = C18:2n-6 + C20:4n-6; Total UFA = Total MUFA + Total n-3 PUFA + Total n-6 PUFA; n-6: n-3 ratio = Total n-6 PUFA : Total n-3 PUFA; UFA: SFA ratio = Total UFA : Total SFA; PUFA: SFA ratio = (Total n-3 PUFA + Total n-6 PUFA) : Total SFA

formulation did not adversely affect the growth performance and feed efficiency of Boer goats.

Fatty Acid Composition of LD Muscle

In this study, the effects of TMR with different levels of lupin inclusion on the fatty acid composition of LD in Boer goat were evaluated.

The predominant fatty acids in the meat of Boer goat LD are oleic (C18:1 42.70%-44.20%, 41.03%-44.34% and 42.96%-44.20% in CON, TMR A and TMR B, respectively), palmitic (C16:0; 19.97%-23.05%, 20.20%-20.79% and 19.74%-21.27% in CON, TMR A and TMR B, respectively) and stearic (C18:0 in CON 10.31%-12.27%, TMR A 10.47-12.30% and TMR B 11.46-13.04%.

In LD, the proportions of lauric (C12:0), palmitoleic (C16:1) and linolenic (C18:3n-3) acids were significantly affected ($p < 0.05$) by the experimental diets, however the total SFA, mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) were unaffected (Table 4). The proportion of lauric acid in TMR B ($0.94 \pm 0.12\%$) was significantly higher than those in CON ($0.65 \pm 0.08\%$) and TMR A ($0.56 \pm 0.07\%$). Whilst, CON had a higher proportion of palmitoleic acid ($3.77 \pm 0.29\%$) than that of TMR A ($3.15 \pm 0.10\%$) and TMR B ($3.07 \pm 0.14\%$). The proportion of linolenic acid was higher in TMR B ($0.76 \pm 0.09\%$) than that of CON ($0.42 \pm 0.07\%$), while its proportion in TMR A ($0.57 \pm 0.11\%$) was indifferent to these groups.

CONCLUSION

Present study showed that the TMR feed with lupin inclusion did not affect the growth performance and meat quality traits of the animals, except the TFI. Reduction in TFI and intramuscular fat of the meat was observed in the animals fed on TMR B (30% lupin). However, similar performance was observed in the animals fed on TMR A (10% lupin) as compared to those fed with a basal diet.

The experimental diets altered the proportions of some of the fatty acids in the meat, however the total SFA, MUFA and PUFA as well as the n-6: n-3 and PUFA:SFA ratios were unaffected. It is therefore shown that the TMR feed is suitable for intensive farming of goats and lupin grain can serve as an alternative protein source to the animals.

Subsequently, the level of inclusion of lupin in the TMR diet was also subjected to economic factors. Considering the dynamic nature of lupin and other common sources of protein and energy prices such as soybean meal, wheat pollard or palm kernel expeller, feed cost should be analysed prudently to ensure the most cost effective using least cost formulation of TMR could be achieved. Better demand for chevon can be expected when consumer awareness is developed to enable them to identify the unique health and organoleptic quality of chevon. Further research should consider other factors that could improve feed intake and feed conversion efficiency of diets containing lupin such as palletisation, heat treatment, various TMR formulation with combination of different locally available ingredients, concentrate feed and fresh fodder.

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