Effect of dietary inclusion of purslane on performance and content of fatty acids in meat of growing rabbits

Efeito da inclusão dietética de beldroegas no desempenho e conteúdo de ácidos graxos da carne de coelhos em crescimento

Efecto de la inclusión de verdolaga en el rendimiento y contenido de ácidos grasos en carne de conejos en crecimiento

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ABSTRACT

This research was carried out in order to assess the effect of including Purslane (*Portulaca oleracea*) on the performance of growing rabbits and meat´s fatty acids profile. Twenty Californian rabbits (1.28 ± 0.081 kg LW) were used in a completely randomized design for 49 days. The treatments were: control diet (with no purslane) and diet with purslane. Rabbit weight gain, feed conversion ratio, carcass yield and the fatty acid profile of the carcass meat were measured (palmitic, stearic, oleic, linoleic and α-linolenic acids). As results higher dry matter intake ($p<0.001$) was obtained with the control diet (4.49 *vs.* 3.47 Kg, respectively), promoting a higher estimated total digestible energy (DE) intake in control diet ($p<0.001$) (58.48 vs 48.67 MJ, respectively), however weight gain, feed conversion ratio and carcass yield were similar for rabbits fed both diets ($p>0.05$) (0.89 *vs.* 0.78 kg). In spite fatty acids intake was similar for both diets ($p>0.05$) (618.62 *vs*. 598.06 mg/ Kg DM) except for stearic acid content of meat which was higher ($p<0.05$) in diet with purslane. Purslane (*Portulaca oleracea*) can be fed to rabbits up to 30% of diet promoting acceptable growth rates, feed conversion ratio and carcass yield for tropical conditions. Purslane inclusion in rabbit diets had little effect on fatty acid profile; However further research with purslane should explore interactions among higher levels of inclusion and longer feeding periods.

Key words: animal feeding**,** animal growth, Oryctolagus, Portulaca oleracea ,

RESUMO

Esta pesquisa foi realizada para avaliar o efeito da inclusão de beldroega (*Portulaca oleracea*) sobre o desempenho de coelhos em crescimento e perfil de ácidos graxos da carne. Vinte coelhos da raça Califórnia foram utilizados em delineamento inteiramente casualizado durante 49 dias. Os tratamentos foram: dieta controle (sem beldoegras) e dieta contendo beldoegras. Foram avaliados o ganho de peso, o consumo de ração, a conversão alimentar, o rendimento de carcaça e o perfil de ácidos graxos da carne (ácidos palmítico, esteárico, oléico, linoléico e α-linolênico). O consumo de matéria seca e de energia digestível foram maiores ($p<0,001$) com a dieta controle (4,49 vs. 3,47 kg e 58,48 vs 48,67 MJ, respectivamente para matéria seca e energia digestível), porém o ganho de

peso, a conversão alimentar e o rendimento de carcaça não variaram (p> 0,05) em função das dietas. O teor de ácidos graxos foi semelhante para as duas dietas (p> 0,05) (618,62 vs. 598,06 mg / kg de MS de carne), exceto para o teor de ácido esteárico que foi maior (p <0,05) na dieta contendo beldoegras. Concluiu-se que a beldroega (*Portulaca oleracea*) pode ser usada na alimentação de coelhos em até 30% da dieta por promover bom desempenho produtivo, apesar de ter tido pouco efeito no perfil de ácidos graxos da carne. No entanto mais pesquisas com beldroegas são necessárias para explorar as interações entre os maiores níveis de inclusão e períodos de ingestão mais prolongados.

Palavras chave: alimentação animal, crescimento dos animais, *Oryctolagus*, *Portulaca oleracea*

RESUMEN

El presente estudio fue realizado para evaluar el efecto de incluir verdolaga (*Portulaca oleracea*) en el comportamiento productivo de conejos en crecimiento y el perfil de ácidos grasos en la carne. Veinte conejos de raza California (1.28 ± 0.081 kg LW) fueron utilizados en diseño completamente al azar por 49 días. Los tratamientos fueron: dieta control (alimento a base de granos) y dieta con verdolaga (0.70 control + 0.30 harina de verdolaga). Se midieron la ganancia de peso, consumo de alimento, conversión alimenticia y el perfil de ácidos grasos (Palmítico, esteárico, oleico, linoléico y α-linolénico) en la canal. Como resultados se observó mayor consumo de materia seca (p<0.001) con la dieta control (4.49 vs 3.47 kg, respectivamente), promoviendo mayor consumo estimado de energía digestible (ED) en la dieta control (p<0.001); sin embargo la ganancia de peso, conversión alimenticia y rendimiento en canal fue similar entre tratamientos (p>0.05). A pesar de que el consumo de ácidos grasos fue similar entre tratamientos (p>0.05) el contenido de ácido esteárico en la carne fue mayor en la dieta con verdolaga (p<0.05). La ganancia diaria de peso y rendimiento en canal fue similar entre tratamientos (p>0.05). Verdolaga (*Portulaca oleracea*) puede ser incluida hasta 30% en la dieta de conejos promoviendo tasas de crecimiento, conversión alimenticia y rendimiento en canal aceptables para condiciones tropicales. La inclusión de verdolaga en la dieta tuvo poco efecto en el perfil de ácidos grasos; sin embargo investigación posterior con verdolaga debería considerar interacciones entre mayores niveles de inclusión y periodos de alimentación más largos.

Palabras clave: alimentación animal, crecimiento animal, Oryctolagus, *Portulaca oleracea*

Introduction

Livestock are a key commodity for human well-being; their importance is the provisioning of food, incomes, employment, nutrient and risk insurance to mankind is widely recognized (Herrero et al., 2009; Rege et al., 2011). Nowadays the role of animal nutrition in creating foods closer to the optimum composition for long–term human health is likely to become increasingly important as well as the use of local resources in order to substitute grains such as sorghum and soybeans from livestock diets. Some tropical plants

have high biomass yield and ideal chemical composition for inclusion in animal feeding and nutrition. The inclusion of some of these plants would promote beneficial modification of final products such as meat (Lukefahr, 2007). Due to its chemical composition Purslane (*Portulaca oleracea*), has been reported as human food, animal feed and medicinal plant, however is mainly considered a weed (Golshan – Zofoori et al., 2013). Furthermore many biological properties of Purslane such as antibacterial (Bae, 2004) anti-inflammatory and wound healing effect have been reported (Xiang et al., 2006). The ability of rabbits to thrive on forages which are locally available makes rabbit meat production a suitable option for tropical areas (Kariaki and Asare, 2009; Olanguju and Sanusi, 2010), nevertheless rabbit feeding schemes have been traditionally based on grains, which in the tropics, could be replaced by local species in order to avoid competition among animal production and human feeding (Nieves et al., 2008). Therefore the objective of this research was to study the effect of dietary inclusion of Purslane (*Portulaca oleracea)* on the performance of growing rabbits under tropical conditions.

Materials and methods

Study site and animals

The study was carried out in facilities from the University of Yucatan, Mexico, where climate is tropical with summer rains (AW0), and average annual rainfall of 940 mm and average temperature of 26°C, relative humidity range from 65% to 85%. Twenty New Zealand white-breed rabbits, weighing 1.3±0.08 kg in average were used. Animals were housed in individual cages of 40 x 80 cm, equipped with plastic feeders and drinkers and received fresh feed and water *ad libitum*.

Experimental design

A completely randomized design during 49 days was used with two treatments (diets) and ten repetitions. Treatments were: control diet (grain based) and diet with purslane (0.70 control diet + 0.30 purslane meal). Dietary ingredients were: vegetable oil, maize meal, molasses, soybean meal, wheat bran and ground star grass hay (Table 1). Diets were analyzed for chemical composition for dry matter (DM), crude protein (CP) (N*6.25) and ash. Neutral detergent fiber (aNDF) was analyzed with sulfate and without amylase, ash inclusive, acid

detergent fiber (ADF) were also determined (Table 2). Digestible energy was estimated both diets. Diets were presented in mashed form. Prior to the beginning of the experiment animals were dewormed with ivermectin (Petspharma 0.1 ml/kg body weight) subcutaneously.

Table 1. Ingredients and inclusion percentage in experimental diets

Ingredient (kg)	Control diet	Diet with purslane
Vegetable oil	5,00	4,00
Maize	21,00	24,00
Molasses	5,00	5,00
Soybean meal	20,00	4,00
Wheat meal	-	5,00
Wheat bran	19,00	28,00
Purslane meal	-	30,00
Star grass meal	30,00	-
Total	100	100

Table 2. Chemical composition of the experimental diets on dry matter basis

	Control diet	Diet with Purslane
Dry Matter (%)	92	92
Crude Protein (%)	16.29	18.66
NDF (%)	38.43	43.24
ADF (%)	21.52	13.86
Ether extract (%)	6.90	7.13
Ash (%)	6.28	11.32
Digestible energy (MJ/ kg DM)	13.06	13.52
DP:DE	7.33	8.53
Fatty acids content (mg/100g)*	137.5	172.03

*Fatty acid content was estimated according to Gutierrez (1993) Mazhidov (1996); Nunes et al. (2003); Eqbal (2011) and Orhun and Kayihan, (2011)

Productive performance

Dry matter and digestible energy intake (based on feed intake and diet chemical composition), daily weight gain (DWG), and feed conversion rate (FCR) were evaluated at the end of the experimental period. Animals were slaughtered at day 49 of experiment with 24 h fasting. Slaughtering procedure, carcass traits definition and dissection technique were followed as recommended by Blasco and Ouhayoun (1993).

Chemical analyses

For fatty acids analyzes, meat samples were taken from the loin (*Longissimus dorsi*) and the hind leg muscles [*Biceps femoris*] (50 g per muscle). Fatty acid were determined and quantified by gas chromatography using a Varian 450-GC gas chromatographer fitted with a flame ionization detector (FID) and a TR-FAME capillary column (120 m × 0.25 mm i.d., 0.25 µm film thickness thermo P/N:260M166L). Samples were grind and mixed and representative samples (5 g) were taken and kept frozen (-15°C) until they were processed for fatty acids analyses. Meat samples were defrosted and 5 g of mixed samples were weighted for fat extraction. Then 40 ml of methanol and 20 ml of chloroform were added, the sample was extracted in an ultrasonic bath (Branson 5510) for 3 minutes. Subsequently 20 ml of chloroform were added and the sample was extracted in an ultrasonic bath for 3 minutes. After this, 20 ml of water were added and the sample was extracted for 10 min. The mixture was passed through a separating funnel and left standing for 30-45 min. Then the mixture was transferred to a flask and dried in a rotary steam (Buchi R - 114) at a temperature of 40 to 50°C. At the end 2.5 ml of hexane were added and stored in vials.

After fat extraction fatty acids methyl esters (FAMEs) were prepared according to a modification of the methylation of total lipids described by using the following procedure: 50 µl lipid extract was placed in a 5 ml screw caped vial and then mixed with 200 µl of the internal standard margaric acid (C17:0 Sigma co.) and dried in a block heater at 60°C. Then 1 mL of methanol 2 mol/L NaOH was added to the vial, tightly closed and heated at 90°C for 3 minutes in the block heater. The sample was allowed to cool and 1 ml boron trifluoride (14%) in methanol solution was added, tightly closed and heated at 90°C in the block heater. After cooling, 2 mL of n-hexane was added, tightly closed and heated at 90°C for 3 minutes in the block heater. Finally, 2 mL of saturated solution of NaOH was added, mixed well and carried at 90°C for 3 minutes in the block heater. After cooling and separation, an aliquot of the top (n-hexane) layer was taken into a 1.5 mL vial (all reagents were provided from Sigma Co.)

Fatty acid were determined and quantified by gas chromatography using

a Varian 450-GC gas chromatographer fitted with a flame ionization detector (FID) and a TR-FAME capillary column (120 m × 0.25 mm i.d., 0.25 µm film thickness thermo P/N:260M166L). The injection volume was 1µl with a split ratio of 1:5, using Helium as the carrier at 1.3 ml/minute and as make-up gas at 25 ml/minute. Working gradient was set with 280°C as detector temperature, 250°C as injector temperature and 150°C as column temperature, during 0.5 minute; temperature was increased 10°C/minute up to 180°C, 1.5°C/minute from 180 to 220°C and 30°C/ minute from 180°C to 260°C and held for 46.50 minutes. Samples were identified by comparison with retention times of standards: 37-Component FAME Mix (Sigma-Aldrich 47885); α- linolenic acid (Fluka 62160); eicosapentaenoic acid methyl ester (Fluka 17266) and docosahexaenoic acid methyl ester (Fluka 05832).

Results analyses

Results were submitted to ANOVA and means were compared using Fisher LSD test. Statistical analysis was performed using Statgraphics 5 (2000).

Results and discussion

Animal performance

Rabbit fed Purslane diet showed a lower DM intake (3.47 *vs*. 4.49 kg), DE intake (48.67 *vs*. 58.48 MJ) ($p<0.05$) than control diet. However traits such as daily weight gain (15g vs. 18g), feed conversion (5.54 *vs*. 5.2) carcass yield (51.75 *vs*. 49.52%) and fatty acids intake (618.62 *vs*. 598.06 mg/ Kg DM) was similar in both control and purslane diet ($p>0.05$) (Table 3). In this experiment DGW gain, DM intake, feed conversion and carcass yield are lower than reported by Abaza et al. (2010) including 10, 20 and 30 % of Purslane in growing rabbits. The main factor influencing weight gain is related to nutrients balance and intake. Dry matter intake is a feature directly affected by energetic density and fiber level inclusion in the diet, inducing changes in animal performance affecting productive traits (Gutierrez et al., 2003). Nevertheless, DE restrictions do not affect muscle development when dietary protein: digestible energy ratio (DP:DE) is increased in diet (Metzger et al. 2009). High digestible fiber and low-starch diets with low DE concentration have been largely used in order to reduce digestive diseases. Dietary protein

effects on performance, carcass and meat quality have been studied by modifying dietary protein concentration. Changes in DP:DE ratio reveal different protein intakes. DP:DE ratios below optimum values of 10.5 – 11.0 g MJ^{-1} are insufficient to cover daily protein requirements, compromising growth due to muscular protein accretion is suboptimal. Despite in current study DP:DE ratio can be considered lower than optimum ratio in both control and Purslane diet, CP: DE intake was higher in rabbits fed purslane diet (12.73 vs 12.27 g/MJ DE) allowing rabbits to express similar growth rate than reported by Adeyemi and Akanji (2012) in rabbits with forage at *ad libitum* diets; According to Xicato and Trocino (2010) when DE concentration decreases from 10.5 to 8.8 MJ kg^{-1} and dietary CP is maintained at 150 g kg^{-1} with 0.70 digestibility, the DP to DE ratio increases from 10 to 12 g MJ^{-1}. However the low DP:DE ratio in the experimental diets had a negative influence on feed conversion (5.20 and 5.54).

Table 3. Performance of rabbits fed diets including or not Purslane (*Portulaca oleracea*)

	Control	Purslane	Standard deviation	P- value
Initial weight (kg)	1.27	1.29	-	0.2010
Final weight (kg)	2.17	2.07	-	0.2030
Daily weight gain (g)	18	15	0.20	0.2044
DM intake (kg)	4.49a	3.47b	0.73	0.0004
CP intake (kg)	0.718	0.62	43.7	0.8548
DE intake MJ	58.48a	48.67b	8.55	0.0063
CP:DE intake (g/MJ DE)	12.27a	12.73b	0.93	0.001
Fatty acids intake (mg/Kg DM)	618.62	598.06	81.24	0.5855
Feed conversion ratio	4.98	4.44	1.06	0.4883
Carcass yield (%)	51.75	49.52	2.68	0.0601

Means followed by different letters in the same row are different by Fischer test ($p<0.05$)

Diets did not affected ($p<0.05$) the fatty acids content in the meat, except by the stearic acid that was higher in meat from rabbits fed diets with purslane (Table 4).

Table 4. Fatty acids profile in meat of rabbits fed diets including or not Purslane (*Portulaca oleracea*)

Fatty acid	Control (g/100 ml)	Diet with Purslane (g/100 ml)	Standard. Deviation.	P-value
C: 16 (Palmitic)	20.44	21.78	1.98	0.0657
C: 18 (Stearic)	7.12a	9.44b	2.34	0.0051
C:18:1N9C (Oleic)	29.38	25.52	8.09	0.2050
C:18:2N6C (Linoleic)	37.63	37.93	2.52	0.7518
C:18:3N3(α linolenic)	2.90	3.12	0.53	0.2563

Means followed by different letters in the same row are different by Fischer test ($p<0.05$)

Meat fatty acid profile

In this study, experimental diets had little effect on meat fatty acids profile ($p>0.05$), stearic acid increased ($p<0.05$) in meat of animals fed the purslane diet. Similar results were found by Forrester – Anderson et al. (2006) and Cavani et al. (2004) in rabbits reared in either outdoor pens or indoor cages with a forage-based diet, increasing stearic and palmitic acid content. According to Pla et al. (2006) in grass based systems or when fibrous diets are used, it is common to obtain leaner carcasses, with significantly lower total fat and a better n-6: n-3 ratio, resulting in a meat with improved nutritional characteristics. Meanwhile Forrester – Anderson et al. (2006) mention that lower fat content and fatty acid profile in carcass meat from animals fed fibrous diets, could be explained by a lower DE content. According to Carrilho et al. (2009) the fatty acid composition of fat depot will reflect the relative contribution from these sources, nevertheless differences in the fatty acid composition of the diet have been more important than differences in the energy content when studying the intra-muscular fatty acid profile. Chillard et al. (2008) notes that this relationship is stronger in monogastrics (pigs, poultry, and rabbits) than in ruminants, where dietary fatty acids are hydrogenated in the rumen. On the other hand, Forrester – Anderson et al. (2006) points that the increase in stearic and palmitic acids has been related to the depression in stearoyl CoA desaturase activity (SCD) which is an endoplasmic reticulum enzyme that

catalyzes the biosynthesis of monounsaturated FA from saturated FA that is either synthesized the novo or derived from the diet. Dobrzyn and Ntambi (2004) mention that SCD activity increases when a high carbohydrate diets is provided, promoting fat deposition, on the other hand in forage based diets (rich in polyunsaturated fatty acids) activity of this enzyme promotes fat catabolism. In rabbits only a low SCD activity has been observed in the liver according to Betnamane et al. (2011). Therefore the potential to modify their α-linolenic acid content through the dietary inclusion of purslane seems to be limited in rabbits.

Conclusion

Inclusion of 30 % of Purslane (*Portulaca oleracea*) in growing rabbit diets promotes low dry matter intake without affecting traits such as growth rate and feed conversion. Purslane inclusion in growing rabbit diets had little effect on meat fatty acid profile; However further research with purslane should explore interactions among levels of inclusion and the assessment length to fully assess physiological aspects of fat synthesis and deposition in the rabbit carcass.

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