Effect of the rate of inclusion of field pea in the concentrate of lambs on in vitro fermentation parameters

Lobón M.S., Joy M., Rufino-Moya P.J., Blanco M.

in

López-Francos A. (ed.), Jouven M. (ed.), Porqueddu C. (ed.), Ben Salem H. (ed.), Keli A. (ed.), Araba A. (ed.), Chentouf M. (ed.).
Efficiency and resilience of forage resources and small ruminant production to cope with global challenges in Mediterranean areas

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 125

2021
pages 475-478

Effect of the rate of inclusion of field pea in the concentrate of lambs on *in vitro* fermentation parameters

M.S. Lobón, M. Joy, P.J. Rufino-Moya and M. Blanco*

Unidad de Producción y Sanidad Animal. Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Instituto Agroalimentario de Aragón (IA2) (CITA-Universidad de Zaragoza)
Avda. Montañana 930, 50059, Zaragoza (Spain)
*e-mail: mblanco@aragon.es

Abstract. The replacement of soya by local protein sources, as the field pea (*Pisum sativum*), has been encouraged to reduce the dependency of Europe on soya imports. The aim of this study was to investigate the effect of increasing amounts of field pea in the lamb's concentrate (0%Pea, 10%Pea, 20%Pea and 30%Pea) on *in vitro* fermentation. Gas production was determined with the Ankom system for 24 hours and the parameters of kinetics of fermentation were estimated. Methane, ammonia (NH_3-N) and *in vitro* organic matter degradability (IVOMD) were determined. Most of the parameters were affected by the rate of inclusion of pea. The 10%Pea concentrate had the lowest gas production and the NH_3-N content ($P<0.05$). Methane production was lower in 10%Pea than in 30%Pea ($P<0.05$), presenting 0%Pea and 20%Pea intermediate values. Regarding the IVOMD, the 10%Pea had greater degradability than 20%Pea and 30%Pea ($P<0.05$) presenting 0%Pea intermediate value ($P>0.05$). In conclusion, a partial substitution of soya by 10% field pea in lamb's concentrate could be recommended because it reduced gas and methane production and NH_3-N content whereas it increased IVOMD.

Keywords. Pea – Degradability *in vitro* – Ovine – Methane – Ammonia.

***Effet du taux d'inclusion de pois de grande culture dans le concentré d'agneaux sur les paramètres de fermentation* in vitro**

Résumé. *Le remplacement du soja par des sources de protéines locales, comme le pois de grande culture (Pisum sativum), a été encouragé afin de réduire la dépendance de l'Europe vis-à-vis des importations de soja. Le but de cette étude était d'étudier l'effet de l'augmentation des quantités de pois (0%de Pois, 10% de Pois, 20% de Pois et 30% de Pois) dans le concentré d'agneau sur la fermentation in vitro. La production de gaz a été déterminée avec le système Ankom pendant 24 heures et les paramètres de cinétique de fermentation ont été estimés. Le méthane, l'ammoniac (NH_3-N) et la dégradation de la matière organique in vitro (IVOMD) ont été déterminés. Le taux d'inclusion du pois a eu un effet sur la plupart des paramètres. Ainsi, le concentré de 10% de Pois a réduit la production de gaz et la teneur en NH_3-N par rapport au reste des concentrés ($p<0,05$). La production de méthane était plus faible pour le concentré de 10% de Pois que chez celui de 30% de Pois ($P<0,05$). Concernant l'IVOMD, le concentré de 10% de Pois avait une digestibilité supérieure à ceux de 20% et de30% de Pois ($p<0,05$) et similaire à celui de l'0% de Pois ($p> 0,05$). En conclusion, une substitution partielle du soja par 10% de Pois dans les concentrés d'agneau pourrait être recommandée car elle réduit la production de gaz, demethane et la teneur en NH_3-N, alors qu'elle augmenterait l'IVOMD.*

Mots-clés. *Pois – Dégradation* in vitro *– Ovine – Methane – Ammoniac.*

I – Introduction

In recent years, an important objective of European countries has been to reduce the use of soybean as protein source in animal diets, promoting the use of alternative protein sources, preferably from local feedstuffs. Most of the soybean is imported and it is genetically modified which causes rejection in part of the European consumers.

The substitution of soya by field pea (*Pisum sativum*), a local source of protein, in the fattening diets of lambs has been encouraged to reduce soya imports. Field pea is an interesting and promising legume crop in Mediterranean areas could potentially be rotated with cereal crops. It is characterized by having high crude protein (25-26% of dry matter), easily degradable in the rumen as all legume seeds (Goelema *et al*., 1998) and high levels of lysine and methionine (Saastamoin *et al.,* 2013). Pea also has high content of starch, with lower ruminal degradability than barley (Walhain *et al.,* 1992). There are few data available on the effects of feeding peas on degradability. Therefore, the aim of this study was to investigate the effect of increasing amounts of pea in the concentrate (0, 10, 20 and 30%) on *in vitro* fermentation parameters, through the *in vitro* gas production technique.

II – Materials and methods

Four concentrates for fattening lambs with different rate of inclusion of pea were evaluated in *in vitro* assay. The rate of inclusion of field pea was 0% (0%Pea), 10% (10%Pea), 20% (20%Pea) and 30% (30%Pea). All concentrates were formulated to be isoenergetic (1.18 MJ/kg FM) and isoproteic (175 g CP/kg FM).The ingredients and chemical composition are presented in Table 1.

Table 1. Main ingredients and chemical composition of the concentrates used in the trial

	Inclusion of field pea in the concentrates			
	0%	**10%**	**20%**	**30%**
Ingredients, %				
Barley	27.3	23	15.5	11.4
Corn	25.7	15	7.5	9.2
Soya meal	22.4	17.5	13	10
Common wheat	20	20	25	30
Pea 22/11	0	10	20	30
Bran	0	8.5	12.8	6.1
Cane molasses	1.5	1.5	1.5	0
Calcic carbonate	1.5	1.5	1.2	1.3
Palm oil	1	2.4	2.9	1.4
Salt and Vitamin	0.6	0.6	0.6	0.6
Chemical composition, g/kg DM				
Organic matter	948 ± 1	947 ± 1	949 ± 1	946 ± 2
Crude protein	199 ± 3	195 ± 4	196 ± 3	188 ± 3
Neutral detergent fibre	238 ± 41	246 ± 47	245 ± 35	252 ± 50
Acid detergent fibre	46.2 ± 2.9	54.8 ± 4.4	62.9 ± 3.7	61.4 ± 2.6
Acid detergent lignin	3.6 ± 2.0	5 ± 2.4	7.8 ± 2.2	5.6 ± 2.2

The analyses of *in vitro* gas production were carried out using the Ankom system (Ankom Technology, NY, USA). Rumen digesta collected from four rumen fistulated wethers, that were fed alfalfa hay:barley grain in a proportion of 70:30, was immediately strained through four layers of cheesecloth. Rumen fluid was mixed with the buffer solution, based on the protocol of Menke and Steingass (1988) in a proportion 1:2 (v/v) under constant CO_2 flux. Three runs were conducted on three separate days. In each run, three sub-samples (0.5 g DM) of each diet were incubated in bottles with 60 ml of incubation solution in a water bath at 39 ºC. Blanks were included in each run and gas production was corrected with the blanks. After 24h of incubation, the bottles were allocated on ice to stop the fermentation (5-10 minutes). Then were tempered at room temperature (10-15 minutes) and a sample of gas produced was transferred into vacutainer tube to determine CH_4 (Rufino-Moya *et al*., 2019). At the end of gas sampling, the pH was measured immediately

with a microPH 2002 (Crison Instruments S.A., Barcelona, Spain). For NH_3-N determination, 2.5 mL of liquid was mixed with 2.5 mL HCl 0.1 N and was determined in Epoch microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) using the colorimetric method described by Chaney and Marbach (1992). The gas production recorded hourly for 24 h by the Ankom system was used to estimate the parameters of the kinetics of fermentation, adjusting the gas produced to the model described by France *et al*., (2000): $P= A*(1-e^{-ct})$, where P is the cumulative gas production (mL) at time t (h), *A* is the potential gas production (mL), and *c* is the rate of gas production (h^{-1}). Methane was analysed with gas chromatograph HP-4890, equipped with a capillary column TG-BOND Q+ (Thermo Scientific). Methane identification was based on the retention time as compared with the standard. *In vitro* organic degradability was estimated by filtering residues using pre-weighed bag (50 ! m; Ankom, NY, USA). The bags with sample were dried at 103 ºC for 48 h to obtain the dry matter content. After 48 h, bag content was weighed and was placed at 550 ºC for to obtain the ashes. The organic matter (OM) of bag content was obtained as DM-ashes and the IVOMD was obtained as: (Incubated OM-bag content OM)/Incubated OM.

Data were analyzed using statistical software SAS V.9.3 (SAS Inst. Inc., Cary, NC, USA). The fermentation kinetics parameters (*A* and *c*) were estimated through a non-linear regression model (Y=a + b*X) using SAS NLIN program. The pH, NH_3-N, total gas, methane, *A*, *c*, and IVOMD were tested by analyses of variance using the GLM procedure of SAS.

III – Results and discussion

The pH was similar among treatments (P>0.05) (Table 2). Most of the parameters were affected by the rate of pea inclusion. Gas production was lowest for the 10%Pea concentrate (P<0.05). The average values of gas production recorded was higher than those observed by Bastida Garcia *et al*., (2011) evaluating field pea hay, probably due to the lower degradability of hay compared with concentrates. The potential gas production (*A*) was greater for 0%Pea and 30%Pea than 10%Pea (P<0.01), presenting intermediate values the 20%Pea concentrate. The lack of a lineal effect with the inclusion of Pea could be related with the different inclusion of others ingredients (corn, barley…) because they were modified to obtain an isoenergetic and isoproteic concentrates. The rate of gas production (*c*) was not affected by the inclusion of field pea (P>0.05).

Table 2. Effect of inclusion of increasing proportion of field peas in the fattening concentrate of lambs on the gas and methane production at 24 hours

	Inclusion of field pea				s.e.m.	P-value
	0%	10%	20%	30%		
pH final	6.59	6.56	6.59	6.56	0.0	0.30
Gas production, ml/g OM	192.8a	179.4b	194.7a	201.7a	11.0	0.012
Gas production kinetics						
A, mL	85.5a	80.4c	82.3bc	85.3ab	3.0	0.003
c, h^{-1}	0.113	0.114	0.123	0.119	0.0	0.18
Methane production, ml/g OM	9.2ab	8.1b	8.6b	9.8a	0.9	0.02
Methane: Gas, %	4.7	4.4	4.4	4.8	0.4	0.14
NH3-N, mg/l	132.2a	111.8b	135.5a	129.9a	13.9	0.02
IVOMD, %	91.5ab	92.3a	88.8c	90.3bc	1.5	0.002

A: potential gas production; *c*: rate of gas production. IVOMD: *in vitro* organic matter degradability; s.e.m.: standard error. Means with different letter differ at P<0.05.

The 30%Pea concentrate had greater methane production than the 10%Pea and 20%Pea concentrates ($P<0.05$). The ratio methane:gas production was not affected by the rate of pea inclusion in the concentrate ($P>0.05$). The content of NH_3-N was affected by the inclusion of field pea in the concentrate ($P<0.05$), presenting the 10%Pea concentrate the lowest value.

The organic matter degradability were also affected by the inclusion of field pea ($P<0.01$, Table 2). The 10%Pea concentrate presented greater IVOMD than the 20%Pea and 30%Pea concentrates, but the 0%Pea concentrate had intermediate value. In contrast, González Garcia *et al*. (2017) did not observe differences in the IVDMD with the inclusion of 0, 25, 50 and 75% of pea replacing soya meal in lamb concentrates. The lack of agreement could be due to the time of incubation which was 96 h in the abovementioned study whereas in the present study the samples were incubated 24 h.

IV – Conclusions

Based on the results from our research it can be concluded that soya can be partially replaced with pea in concentrates of fattening lambs. The use of the concentrate with an inclusion of 10% of field pea would be the most recommended rate, because it reduced gas and methane production and ammonia content whereas it improvement IVOMD.

Acknowledgments

The authors gratefully acknowledge the staff of the CITA Research Centre for technical support, especially to Angelines Legua and Andrés Domínguez. Project RTA2014-00032-c02-01 and RZPP2017; INIA and ESF funded M. Blanco contract.

References

Bastida Garcia J.L., González-Ronquillo M., Domínguez Vara I.A., Romero-Bernal J. and Castelán Ortega O., 2011. Effect of field pea (Pisum sativum L.) level on intake, digestion, ruminal fermentation and in vitro gas production in sheep fed maintenance diets. *Animal Science Journal* 82: 654-662.

Chaney A.L. and Marbach E.P., 1962. Modified reagents for determination of urea and ammonia. *Clinical Chemestry, 8*, 130-132.

France J., Dijkstra J., Dhanoa M.S., Lopez S. and Bannink A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: Derivation of models and other mathematical considerations. *British Journal of Nutrition* 83: 143-150.

Goelema J.O., Spreeuwenberg M.A.M., Hof G., Van Der Poel A.F.B. and Tamminga S., 1998. Effect of pressure toasting on the rumen degradability and intestinal degradability of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Animal Feed Science and Technology* 76: 35-50.

González Garcia U.A., Corona Gochi L., Estrada Flores J.G., Abarca Amesquita D.K. and González Ronquillo M., 2017. Ruminal and intestinal digestion of maize (Zea mays) and sorghum (Sorghum bicolor L. Moench) using different degradability techniques (*In vivo*, *in vitro* and *in Sacco*)]. *Tropical and Subtropical Agroecosystems* 20: 183-194.

Menke K.H. and Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* 28: 7-55.

Rufino-Moya J.P., Blanco M., Bertolín J.R. and Joy M., 2019. Effect of the method of preservation on the chemical composition and *in vitro* fermentation characteristics in two legumes rich in condensed tannins. *Animal Feed Science and Technology* 251: 12-20.

Saastamoinen M., Eurola M. and Hietaniemi V., 2013. The chemical quality of some legumes, peas, fava beans, blue and white lupins and soybeans cultivated in Finland. *Journal of Agricultural Science and Technology* B3(3): 92-100.

Walhain P., Foucart M. and Théwis A., 1992. Influence of extrusion on ruminal and intestinal degradability in sacco of pea (Pisum sativum) proteins and starch. *Animal Feed Science and Technology* 38: 43-55.