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EFFECT OF STARCH SUBSTITUTION WITH CRUDE GLYCEROL ON GROWING RABBIT AND LACTATING DOE PERFORMANCE

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Abstract: The aim of this work was to study the effect of dietary inclusion of 2.5 or 5.0% of glycerol in substitution for starch on performance of lactating does and fattening rabbits. Over 4 consecutive reproductive cycles, a total of 81 crossbred rabbit does and 813 young rabbits weaned at 25 (fattening trial 1) or 35 d of age (fattening trial 2) were allocated at random to the experimental treatments. Inclusion of glycerol in the diet up to 5% did not influence total feed consumption of does and suckling rabbits, body weight and bioelectrical impedance of does at parturition or at 21 d of lactation and litter weight at weaning, or reproductive efficiency. Substitution of starch with glycerol did not affect feed intake, weight gain or mortality during fattening. The results of the current study indicate that crude glycerol from the biofuel industry can be used at levels up to 5% in rabbit diets without any detrimental or beneficial effect on performance.

Key Words: crude glycerol, biofuels, lactating does, fattening rabbits, performance.

INTRODUCTION

Glycerol is the principal co-product of the increasingly important production of biodiesel from vegetable oils and animal fats, at a rate of around 10 L to every 90 L of biodiesel obtained. The process includes transesterification, in which the glycerol content of the triglycerides is substituted with methanol, using NaOH or KOH as catalyst and ClH or SO_4H_2 as acidifiers. The commercial product (crude glycerol) marketed for the animal feed industry contains 8-20% moisture, 2-10% minerals and 70-93% glycerol (FEDNA, 2010).

Pure glycerol contains about 18.0 MJ/kg of gross energy (Lammers *et al*., 2008a), 16.1 and 15.4 MJ/kg of digestible and metabolisable energy for growing pigs, respectively (Lammers *et al*., 2008b), and 15.9 and 16.5 MJ/kg of N-corrected metabolisable energy for laying hens (Lammers *et al*., 2008a) and broiler chickens (Dozier *et al*., 2008), respectively. These values are around 10% higher than those assigned to corn grain in these species. Moreover, a high proportion of glycerol (>97%, Lammers *et al*., 2008b) is digested prior to the caecum in growing pigs. Its high absorption rate is likely due to its small molecular size and high water solubility. Once absorbed, glycerol can be easily converted to glucose in the liver. Substitution of starch with glycerol in

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rabbit diets might then reduce the carbohydrate flow to the hindgut and could decrease microbial and pathogen proliferation, without modifying glucose availability for metabolism.

However, the possibility of using crude glycerol as a rabbit feed supplement is unknown. High levels (>10%) of glycerol inclusion in the diet have been associated with an increase in urinary losses of glycerol in fattening pigs (Kijora *et al*., 1995; Doppenberg and Van Der Aar, 2007; Lammers *et al*., 2008b) and with an impairment of growth performance in broilers (Cerrate *et al*., 2006) and fattening pigs (Della Casa *et al*., 2009). Furthermore, ingestion issues should be assessed before recommending commercial use of crude glycerol. The purpose of this research was to determine the effects of crude glycerol supplementation in isoenergetic and isoproteic diets for lactating does and growing rabbits on intake, feed efficiency and productive performance.

MATERIAL AND METHODS

Animals were handled according to the principles for the care of animals in experimentation published by Spanish Royal Decree 1201/2005 (BOE, 2005).

Diets

A basal diet was formulated according to the nutrient recommendations of De Blas and Mateos (2010) for compound feeds for both breeding does and fattening rabbits. Another 2 diets were formulated by adding 2.5 or 5.0 % of crude glycerol in substitution of starch on an isoenergetic, isofibrous and isoproteic basis. Crude glycerol contained 9.2% moisture, 4.3% ash, 85.6% of glycerol, in addition to 1.5% sodium and 2.8% chloride, so that sodium chloride inclusion in the experimental diets containing crude glycerol was reduced compared with the control diet. The ingredient and chemical composition of the experimental diets is shown in Tables 1 and 2, respectively. Rabbits had *ad libitum* access to feed and water during the whole trial. Neither feed nor drinking water were medicated with antibiotics. However, a coccidiostat (diclazuril) was added to all the experimental feeds.

Lactation trial

Eighty-one crossbred rabbit does (originating from strains Universitat Politècnica de València, Spain) were assigned at random to the 3 experimental diets and controlled 4 consecutive reproductive cycles. Rabbit does were inseminated at 11 d post-partum and their litters were weaned at 25 or 35 d of age. Culled rabbit does were replaced immediately.

Rabbit does were housed individually in flat-deck cages measuring 600×500×330 mm high. Heating and forced ventilation systems allowed the building temperature to be maintained between 18 and 23ºC during the trial. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Litter size was standardised at kindling within dietary treatments. Feed intake, prolificacy (total number of rabbits born and stillborn) and mortality of the young rabbits during lactation and litter weight at weaning were measured per rabbit doe and cycle. Body weight and bioelectrical impedance (BIA) of does was determined at parturition and at 21 d of the lactation period (Pereda, 2010). Impedance was measured to estimate body fatness using a four-terminal body composition analyser (Model Quantum II, RJL Systems, Detroit, MI, USA) which reports reactance and resistance between 2 sets of 2 electrodes. Standard 21-gauge needles (Terumo Europe N.V. 3001 Leuven, Belgium) were used as electrodes, inserted into the skin of the animal. One pair of needles was held 1 cm apart in the neck and the other in the rump.

Table 1: Ingredient composition of the experimental diets as the percentage added of Glycerol (% as fed basis).

	Glycerol		
	0	2.5	5
Wheat grain	13.4	9.8	6.2
Wheat bran	32	32	32
Sugarcane molasses	2.5	2.5	2.5
Sugar beet pulp	4	3.5	3
Soybean hulls	5.4	5.1	4.8
Grape seed meal	0.6	0.4	0.2
Wheat straw	1.3	0.9	0.5
Lucerne meal	17	19.4	21.8
Sunflower meal (28%)	13.6	13.6	13.6
Palm kernel meal	3	3	3
Full fat soybean meal	1.6	1.7	1.8
Soybean meal (47%)	1	1.2	1.4
Palm oil	1.5	1.5	1.5
Crude glycerol	0	2.5	5
Calcium carbonate	1.5	1.4	1.3
Monocalcium phosphate	0.48	0.5	0.52
Sodium chloride	0.53	0.43	0.33
Choline chloride	0.04	0.04	0.04
DL-methionine	0.05	0.05	0.05
L-lysine HCl	0.3	0.28	0.26
L-threonine	0.01	0.01	0.01
Clinacox, 5% diclazuril	0.02	0.02	0.02
Vit-min premix[1]	0.17	0.17	0.17

[1]Vitamin and mineral premix supplied for 1 kg of complete diet; Vitamin A: 11.390 IU, Vitamin D_3: 1.360 IU, Vitamin E: 47.6 IU, Vitamin K_3: 1.7 mg, Thiamine: 1.7 mg, Riboflavin: 4.3 mg, Pantothenic acid: 13.6 mg, Pyridoxine: 1.7 mg, Biotin: 85 µg, Folic acid: 850 µg, Vitamin B_{12}: 13.6 µg, Fe: 47.6 mg, Cu: 17 mg, Zn: 68 mg, Mn: 22.7 mg, Co: 595 µg, Se: 140 µg, I: 1.2 mg.

Fattening trial

Two fattening trials were conducted using rabbits from the third and fourth cycles of the lactation assay, respectively. In trial 1, a total of 312 growing rabbits weaned at 25 d of age were assigned at random to the 3 experimental treatments. Ninety-six rabbits (32 per treatment) were housed individually, whereas the remaining 216 were caged in groups of 3 (24 cages per treatment). In trial 2, a total of 501 rabbits weaned at 35 d of age were used. Ninety-six were housed individually and 405 in groups of 3 (45 cages per treatment). Animals caged individually in both trials were blocked by litter, whereas those housed collectively received during the fattening period the same feed as before weaning. Individual and collective cages measured respectively 300×500×300 and 600×500×300 mm high. Building temperature was partially controlled (average room temperature 20±3ºC). A cycle of 12 h of light and 12 h of dark was used throughout the growth

Table 2: Chemical composition of the experimental diets as the percentage added of Glycerol (%, as fed basis).

	Glycerol		
	0	2.5	5
Dry matter	90.2	90.2	90
Ash	8	8.1	8.1
Crude protein	15.5	15.9	15.8
Starch	16.1	14	11.8
Neutral detergent fibre	34.9	34.6	34.3
Acid detergent fibre	16.8	17	17.1
Acid detergent lignin	5	4.9	5.1
Ether extract	2.8	2.8	3
Digestible energy[1] (MJ/kg)	9.11	9.29	9.36
Lysine[2]	0.8	0.8	0.8
Methionine[2]	0.32	0.32	0.32
Threonine[2]	0.58	0.58	0.58
Calcium[2]	1.1	1.1	1.1
Phosphorous[2]	0.62	0.62	0.62

[1]Estimated according to De Blas *et al*. (1992).
[2]Values calculated according to FEDNA, 2010.

trial. Feed intake and average daily gain of the rabbits up to 60 d of age were recorded only in individual cages. Mortality was controlled daily throughout the experimental period both in individual and collective cages.

In both fattening trials, soft faeces samples were taken 10 d after weaning from 7 rabbits per treatment selected at random from those housed in collective cages. To collect soft faeces, animals were placed on individual metabolism cages and fitted with a neck collar which avoided soft faeces re-ingestion. Rabbits weighed at this point 767±137 (standard deviation) g and 1290±202 g on average when weaned at 25 (trial 1) or 35 (trial 2) d, respectively. Collars were made of transparent plastic (33.0 g and 330 mm external diameter on average) and were fitted from 8:00 to 12:00 a.m. *Clostridium perfringens* enumeration in soft faeces was performed as described by Romero *et al*. (2009b) to predict proliferation of this bacteria in the caecum.

Chemical analyses

AOAC procedures (2000) were used to determine the concentrations of dry matter (934.01), ash (967.05), crude protein (968.06), ether extract (920.39), and starch (amyloglucosidase-a-amylase method, 996.11). Dietary neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined sequentially by using the filter bag system (Ankom Technology, New York) according to Mertens (2002), AOAC (2000; procedure 973.187) and Van Soest *et al.* (1991), respectively.

Statistical analyses

Data were analysed using SAS software (Statistical Analytical Systems Institute, 1990). The results obtained in the lactation trial were studied using a repeated measures analysis by the

MIXED procedure (Littell *et al*., 1996), including in the model the type of diet, the parturition number and their interaction. A compound symmetry structure was fitted, as it showed the highest value of the Schwarz Bayesian criterion (Littell *et al*., 1998). It was considered that measures at all times had the same variance, and that all pairs of measures on the same animal had the same correlation. Fertility data were analysed using the GLIMMIX procedure.

Fattening trial data were analysed as a completely randomised design with type of diet as the main factor, using the General Linear Model (GLM) procedure. Litter effect was also included in the model for the traits measured in individual cages. *Clostridium perfringens* counts and fattening mortality results were analysed using generalised linear models with the GENMOD procedure of SAS and considering the cage as the experimental unit.

RESULTS AND DISCUSSION

Lactation trial

The effects of dietary treatments on several productive traits of rabbit does and young rabbits are shown in Table 3. Since no interaction between type of diet and parturition number was observed, the data are presented as average values per treatment throughout the experimental period.

The inclusion of glycerol in the diet up to levels of 5% did not influence total feed consumption (mothers+kits), either in the first 3 wk of lactation or in rest of the whole reproductive cycle. Type

Table 3: Effect of glycerol inclusion level in the diet on performance of lactating rabbit does.

	Glycerol				
	0	2.5	5	SEM	Significance
Initial number of animals	27	27	27		
Daily feed consumption by does and suckling kits (g)					
From 1 to 21 d of lactation	345	327	327	15	NS
From 21 d of lactation to next parturition	384	376	371	18	NS
Rabbit does weight (g)					
Parturition	4070	4037	4060	67	NS
21 d after parturition	4438	4401	4330	73	NS
Rabbit does bioimpedance (Ω)					
Parturition	107	104	106	3	NS
21 d after parturition	98.1	96.5	96.9	3.9	NS
Fertility, %	75.3	79.2	76.1	4.6	NS
Number of young rabbits					
Born alive	10.9	10.7	10.6	0.5	NS
Born dead	0.925	0.929	0.931	0.005	NS
After standardisation	10	10	10	-	-
Weaned	9.29	9.41	9.04	0.93	NS
Litter weight at weaning (g)	3999	4190	3817	203	NS

SEM: Standard error of means. The interaction diet×number of parturition was not significant (P>0.10) for all of the traits studied. NS: not significant.

of diet had no effect on rabbit does' weight or BIA (which estimates body fat reserves) measured at parturition and 21 d after. Fertility, prolificacy and replacement rates of rabbit does averaged 76.5%, 10.7 and 46.9% during the trial and were not affected by treatments. Main reasons for culling were mastitis and infertility. Mean values obtained for litter size and weight at weaning were slightly lower in the diet containing the highest glycerol level, but differences did not reach significant levels.

A previous work (De Blas *et al*., 1995) had reported an impairment of milk yield, litter weight at weaning and interval between parturitions as a consequence of reducing dietary starch from 17.1% to 11.7% (as fed basis) mainly by replacing it with neutral detergent fibre. However, in the present study no detrimental effects on rabbit does' performance were detected when a similar reduction in dietary starch level was obtained by replacing it with glycerol. Accordingly, glycerol seems to act as a gluconeogenic nutrient able to meet glucose requirements in reproductive rabbit does. Similar results have recently been obtained (Schieck *et al*., 2010) when including up to 9% of crude glycerol in corn-soybean based diets for lactating sows.

Fattening trial

Effects of dietary treatments on growing performance are shown in Table 4. Animals in trial 1 and 2 were weaned at 25 and 35 d of age, respectively. These trials were conducted back to back because of farm management restrictions, although the origin of the animals and housing

Table 4: Effect of glycerol inclusion level in the diet on performance and mortality of fattening period of rabbits from weaning at 25 d (Trial 1) or at 35 d of age (Trial 2) until 60 d.

	Glycerol				
	0	2.5	5	SEM	Significance
Trial 1, individual cages (32 cages/treatment)					
Initial weight (g)	494	482	495	7	NS
Feed intake (g)	115	114	121	2	+
Daily weight gain (g)	46.4	46.2	48.6	0.8	+
Feed conversion rate (g/g)	2.49	2.48	2.49	0.03	NS
Mortality (%)	3.12	12.5	0	-	NS
Trial 1, collective cages (24 cages/treatment)					
Mortality, %	12.5	9.72	13.9	-	NS
Clostridium perfringens counts (cfu×10^3/g)	204	81	247	-	NS
Trial 2, individual cages (32 cages/treatment)					
Initial weight (g)	847	856	853	6	NS
Feed intake (g)	135	140	139	2	NS
Daily weight gain (g)	53.7	54.1	53.7	0.7	NS
Feed conversion rate (g/g)	2.5	2.59	2.58	0.03	NS
Mortality (%)	6.25	3.1	0	-	NS
Trial 2, collective cages (45 cages/treatment)					
Mortality (%)	2.76	0.69	1.38	-	NS
Clostridium perfringens counts (cfu×10^3/g)	2.61	1.02	4.53	-	NS

SEM: Standard error of means. + P<0.10; NS: not significant.

conditions were the same. A higher mortality was observed in early weaned animals housed in collective cages (12.0 *vs.* 1.60%) in parallel to higher *Clostridium perfringens* counts in the soft faeces (177×10^3 *vs.* 2.7×10^3 cfu/g). These results agree with previous works showing that a delayed weaning age increased young rabbits viability and reduced *E. coli* O103 (Gallois *et al*., 2007) and Cl. perfringens (Romero *et al*., 2009a) proliferation in faeces and caecal contents, respectively.

Early weaned rabbits fed the diet with the highest glycerol content in trial 1 had no significant higher values for feed intake (P=0.09) and average daily gain (P=0.07) than those fed the other diets, so that feed conversion was not modified. These effects were not observed when animals were weaned at 35 d (trial 2). These results suggest that experimental diets in which glycerol replaced starch had a similar nutritive value. They can be explained taking into account that energy content of glycerol was similar to that of starch (18 MJ/kg) and a high energy digestibility (>90% in other monogastric species, when included in the diet at moderate levels; Lammers *et al*., 2008a,b; Dozier *et al*., 2008).

In the current experiment, neither fattening mortality nor *Clostridium perfringens* counts in the soft faeces were affected by starch substitution with glycerol, even although mortality reached moderate levels in some experimental conditions (12% as average in rabbits weaned at 25 d and reared in collective cages).

In conclusion, the results of the current study indicate that crude glycerol can substitute starch up to levels of 5% in rabbit diets with neither adverse nor beneficial effect on feed consumption, feed efficiency and lactating and growing performance.

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