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Report on the SOLID participatory research from Romania: Assessing the effects of inclusion of Camelina meal and grape marc on dairy cows' performances

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Summary

Two on-farm trials were organised at the Romanian SME partner, Agro Solomonescu SRL, in order to assess the effects of two novel / underutilised by-products on the dairy cows' performances. The by-products used were Camelina meal, which results from the oil extraction from the Camelina seeds, and dried grape marc, which results from winery.

The trials were conducted under commercial farm conditions: the animals were distributed in two statistically comparable groups and received two diets: a control diet, formed of feeds that are commonly used by the farmer and the experimental diet, where one or two of the classical feeds were replaced by the studied by-products, either Camelina meal or grape marc, while maintaining the nutritive supplies of the diets (protein, energy, minerals, vitamins).

Camelina meal entirely replaced sunflower meal (1:1 ratio) in a diet based on whole corn green biomass and alfalfa hay, whereas grape marc replaced one third of the cereal grains (1:3.2 ratio) in a diet based on corn silage and alfalfa hay.

At the beginning of the trials, the farm had no possibility to individually record intake and milk production. In February 2014, the farmer upgraded its milking parlour thus allowing the measurement of milk production for each cow. Therefore, individual milk production was recorded periodically for Camelina trial and daily for grape marc trial. Feed and milk samples were taken for proximal crude protein, fat, fibres etc. and, in case of milk, for fatty acids profile.

Replacement of sunflower meal with Camelina meal had no significant effect, neither on milk yield nor on milk protein or lactose. On the other hand, it decreased milk fat content about 15%, which confirm previously reported findings in the literature. The effects on milk fatty acids profile were positive, with an increase of PUFA (including the CLA).

Replacement of one third of corn grains and barley grains with dried grape marc, while maintaining the nutritive supply of the diets did not statistically influenced milk yield and milk composition (milk fat, protein and lactose content). On the other hand, it significantly increased milk PUFA, especially n-6 PUFA and, of these, the linoleic acid.

It is concluded that both Camelina meal and dried grape marc can replace, at least on short term, the more classical feedstuffs without noticeable adverse effects, except the decrease of milk fat in the case of Camelina meal. Overall, positive effects on the quality of milk fat were observed for both by-products. It is important to underline that these results were obtained within a low input production system, on cows having moderate production level. Whether these feedstuffs can be used extensively in the nutrition of the dairy cow will be determined - as expected - by their price in the market.

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1 Aims and research questions

The main objective of the on-farm experiments was to assess the effects of Camelina meal (on-farm trial 1) and dried grape marc (on-farm trial 2) on the productive performances of dairy cows (milk production, milk quality, etc.) and to determine whether these by-products can serve as potential alternative feeds for ruminants. The availability of both by-products is likely to increase in the future due to the increased biofuel production and because there is a need for the vinery industry to discharge these specific "wastes" (i.e. grape marc).

2 Background

2.1 Farmer background

The two on farm experiments were shared between WP1 and WP3, having therefore two objectives:

a) Ensuring participatory research within WP1, by involvement of the farmers in all stages of RD activity: identifying the research priorities, choice of solutions to be tested, organisation of the experiments, running the experiments, analysing and interpreting the results, dissemination and demonstration activities.

b) Valorisation of the experiments previously done in WP3 (biochemistry, in situ degradability, tests on fistulated animals) by assessing the effects of the proposed solutions in real production conditions (on farm), thus ensuring direct applicability of the results.

The research priorities were identified in earlier stages of the project, during the Rapid Assessments of dairy farms and farmers' workshops (also WP1 activities). The discussions revealed that, with few exceptions, industrial by-products are insufficiently known and used by the farmers. However, farmers declared themselves open to try these feeds, if they were recommended by researchers. From the farmers' point of view the distinction between a need for knowledge transfer and a need for research is difficult, as they do not know what research has already been done. Therefore, the choice of the feeds to be studied was also based on a literature review which revealed knowledge gaps related to these by-products. An extensive list of by-products was discussed and two feeds were chosen on the basis of identified knowledge gaps, farmers' acceptance and availability for purchasing: **Camelina meal** and **dried grape marc**.

Camelina meal. The practical interest is related to the increasing crop production as Camelina is likely to be used for biofuel production. This results to increased available quantities of a by-product (i.e. Camelina meal), which has a chemical composition that suggests a nutritive potential that is similar to other well-known protein meals, such as sunflower meal or rapeseed meal. On the other hand, there are numerous gaps in the knowledge that make the farmers reluctant to the use of Camelina meal in cow nutrition. Although Camelina is currently a non-improved species, with low yields, it is also a low-input cultivar (resilient, low nutrients requirements, etc.) and it has a good potential of genetic improvement. The farmer's objective was to test whether Camelina meal can replace classical protein meal such as sunflower meal or rapeseed meal in diets, as these feeds have similar protein content.

Dry grape marc. The practical interest of using grape marc in low-input dairy farms is based on its large availability and therefore on its potential as alternative feedstuff, e.g. in case of feed shortages. Although the theoretical nutritive value is not high, the farmer's objective was to test whether it can be used for short-term replacing of classical feeds, without noticeable adverse effects on milk production.

2.2 Research background

Camelina meal (*Camelina sativa*) is a by-product of oil extraction, usually mechanical. Besides the high protein content, it has a variable proportion of residual oil, which adds to its energetic value. The use of this crop is likely to increase in the future (Matthaus et al., 2004; Melcher, 2010) and its potential for organic production systems has already been identified (Henriksen et al., 2008). The nutritive value of Camelina meal varies because of the variation in raw materials and processing technologies, and presumably its potential effects on animal performance will also vary. Camelina meal has a Crude Protein content that varies from 23 to 41% and NDF content from 27 to 35% (Zubr, 2003; Hurtaud & Peyraud, 2007; Malgorzata et al., 2011; Moriel et al., 2011). Camelina meal is abundant in essential amino acids (Halmemies et al., 2011) whereas its residual oil has a high content of polyunsaturated fatty acids, having potential effects on milk quality. So far, only a few studies on using Camelina meal in dairy cows feeding were conducted. Moriel et al., 2011, reported an *in vitro* DM digestibility of 0.706, while Hurtaud & Peyraud (2007) reported shifts in C2:C3 ratio in rumen fluid. There are only a few results on the use of Camelina meal in cattle, two of which focus on the residual oil (Hurtaud and Peyraud, 2007, Halmemies et al., 2011). In both studies, milk yield was not influenced, whereas effects on milk fat were divergent: first team reported a decrease whereas the second team found no effects. Divergent results were obtained also for DMI: no effects were found by Hamelmies et al, 2011 (on dairy cows) and Moriel et al., 2011 (on heifers) whereas Hurtaud and Peyraud reported a decrease, following inclusion of camelina meal in diets. However, several authors referred to changes in FA profile of milk. No studies on the effects on immune system were found. In conclusion, the effects on animal performances are not known for all the main patterns of feeding strategies.

Grape marc. The winery industry produce huge quantities of by-products (Nair & Pullammanappallil, 2003), which raise environmental problems when disposed improperly (Cataneo et al., 2008, cited by Santos, 2014). Although grape marc as a by-product is widely available and its use in animal feeding is not uncommon, the number of studies testing its effects on animal performance is unexpectedly low. The increasing concern about the future availability of feed resources and the more strict rules for environmental protection may stimulate the use of this by-product despite its rather low nutritive value. Grape marc contains an estimated 30% stalks, 23% seeds and 42% peels (Nerantzis şi Tataridis, 2006), but these figures provide an orientation only (while these proportions can influence the nutritive value). Also, the high content of lignified cell walls and tannins is important from a nutritional point of view.

The crude protein content varies between 12.5 and 17% while NDF content varies between 22 and 62%; the digestibility also varies, while its level is generally low (Sauvant et al, 2002; Y. Pétriz-Celaya et al, 2010; Moghaddam et al, 2012). The variability in the nutritive value is likely due to the different raw materials used such as red or white grapes (Zalikarenab et al, 2007) but also to the different processing methodologies. Molina, 2008, found low rumen degradability of both dry mater

and protein, but this advantage is irrelevant in the context of a low protein content and low total tract digestibility.

Only few studies on the effects on animal performances were found. Greenwood et al. (2012) investigated the effects of grape marc on nitrogen partition and found high excretion of nitrogen in faeces. Pétriz-Celaya et al. (2010) reported higher DMI in lambs, presumably due to good palatability of grape marc. Santos et al. (2014), found no effects on DMI or milk production when grape marc was fed up to 10% of the diet (DM basis), as silage. As the number of available studies is low, they are not covering even the main feeding situations, which support the need for further research. In addition, the potential influence of active substances on milk quality and on the immune system also merit further investigation.

3 Methodology and data collection

The general procedure was similar for both feeding trials: statistically comparable groups of animals received diets including Camelina meal (trial 1) or grape marc (trial 2) and a control diet (based on usual feeds in the area) while maintaining equal dietary supplies of nutrients. For at least 12 weeks, feed intake data and milk production were recorded; feed and milk samples were collected periodically and analysed for nutritive value and quality, respectively.

3.1 Location of the farms

Both on-farm feeding trials took place at **Agro Solomonescu farm** (SME partner from Romania), in Miron Costin village, **Botosani county**, **North-East of Romania**.

3.2 Timetable

The experiments took place in July – October 2013 (the trial on Camelina meal) and March – June 2014 (the trial on grape marc).

3.3 Additional data collection

Milk yield, its proximal quality (content of protein, fat and lactose) and milk fatty acids were determined. Also, milk samples were retained for analysing milk protein fractions and blood samples were retained to be analysed for plasma immunoglobulins and total antioxidant capacity in order to assess the effects on immune status (part of WP3).

3.4 Further details of methods

3.4.1 Feeding trial on Camelina meal

The objective of this study was to assess the potential of Camelina meal to replace the more classical sunflower meal in dairy cows diets. This feeding trial was organized in a mono-factorial experimental design and aimed to estimate the effects of Camelina meal particularities (residual oil, fatty acids profile) on milk yield and quality. Preliminary data shows similarities with the rapeseed meal, but there are a series of unknown aspects (e.g. rumen degradability, influence of residual fats, etc.) that have to be studied. The farm was not adapted for experimental activities; therefore the trial had to be conducted in less controlled conditions which means that it was not possible to measure the individual ingesta of the cows. Also, the milk production could not be measured every day for each cow separately. As the farmer has the possibility to distribute the cows in two groups only,

sunflower meal was totally replaced by Camelina meal (no intermediary level of replacement). The diets were formulated in agreement with the farmer, who opted to maintain its usual feeding strategies, while accepting to try the use of Camelina meal.

In the trial 50 Holstein Friesian cows of low production level (~ 14 kg of milk per cow per day), were used and kept in free stalls. The cows were randomly distributed in two statistically comparable groups, which received the same basal diet: whole corn green biomass (chopped, ad libitum) and alfalfa hay (limited amount). The difference between the diets was done by the structure of the concentrates mixtures. The control diet was constituted of barley, oat, pea and sunflower meal (24.2% in the concentrate mixture) while in the experimental diet, the sunflower meal was entirely replaced by Camelina meal (Table 1). The diets had similar nutritive supplies (protein, energy, etc.)

Table 1. The diets in the Camelina meal trial (kg DM / d)

	Control group	Camelina group
Basal diet		
Corn green biomass	7.95	7.95
Alfalfa hay	2.10	2.10
Concentrates mixture		
Barley grains	1.30	1.30
Oat grains	1.74	1.74
Pea	0.86	0.86
Sunflower meal	1.34	0
Camelina meal	0	1.36

After the adaptation to diets, milk production per group was recorded daily, for 12 weeks. Also, individual milk production was recorded periodically. Milk and feeds samples were taken and transported to INCDBNA for biochemistry analyses.

3.4.2 Feeding trial on grape marc

The objective of this study was to assess the potential of dried grape marc to temporarily replace cereals in dairy cows diets, in case of feed shortages, in a low-input system.

For the trial with the grape marc, 30 Holstein Friesian and Fleckvieh cows of a rather low production level (~ 14 kg of milk per cow per day) were used and kept in free stalls. The cows were randomly distributed in two statistically comparable groups, which have received the same basal diet: corn silage (ad libitum) and alfalfa hay (limited amount). The difference between the diets was done by the structure of the concentrates mixtures.

The control diet was constituted of corn, barley and sunflower meal, whereas in the grape marc diet, one third of the corn and barley quantities were replaced by dried grape marc (Table 2). In order to maintain similar nutritive supplies of the diets, the replacement was made in 1:3.2 ratio. This raised questions whether the intake or rumen processes will be negatively influenced and whether the dietary nutritive potential will be indeed reflected in the milk production.

Table 2. The diets in grape marc trial (kg DM / d)

	Control group	Camelina group
Basal diet		
Corn silage	10.23	9.65
Alfalfa hay	2.51	2.51
Concentrates mixture		
Corn grains	0.83	0.56
Barley grains	1.73	1.16
Sunflower meal	1.78	1.78
Dried grape marc	0	2.70

The experimental conditions were similar to those of Camelina feeding trial, therefore individual intake could not be measured; however, in this experiment milk yield was recorded daily (following

researchers' advise, the farmer upgraded its milking parlour in February 2014 in order to allow measurement of milk production for each cow).

4 Results and Discussion

4.1.1 Feeding trial on Camelina meal

The replacement of sunflower meal with Camelina meal had no significant influence on the dry mater intake. However, this result had to be considered with caution, as the farm infrastructure did not allow the individual recording of the intake.

Also, the replacement had no influence on the milk yield – the slight decrease (from 13.09 to 12.40 l/d) was not statistically significant. It is important to mention that the milk yield is lower than the level expected from the dietary supply, which reveal an inefficiency of the valorisation of the diets into milk production.

Table 3. The effect on milk yield and composition - Camelina trial

	Treatment		***SEM***	***P value***
	Control	**Camelina**		
Milk yield, l/d	13.09	12.40	0.512	0.507
% fat	4.070	3.551	0.128	0.038
% protein	3.523	3.385	0.087	0.447
% lactose	4.679	4.543	0.061	0.283

Also, the effect on the milk composition was insignificant, except the effect on milk fat, which decreased from 4.07 to 3.55 ($P = 0.038$). Such decrease, although in a much higher extent, was previously reported by Hurtaud et al., 2007, on a smaller number of animals (fistulated). This decrease may be important in farmers decisions on feeding strategies as in some countries (e.g. Romania) milk fat is the only variable for correcting the milk price at the farm' gate.

The inclusion of Camelina meal also has modified the milk fatty acids composition. The effects were more pronounced for the essential fatty acids. Thus, the α-linolenic acid increased 1.27 times ($P<0.01$) while the concentration of FA n-3 increased 1.76 times ($P<0.001$). Content of some FA has increased even more: 4.8 times the eicosatrienoic acid, 2 times the CLA, etc. Overall, the PUFA increased by 41.81% comparing to the control ($P<0.001$).

4.1.2 Feeding trial on grape marc

The inclusion of dried grape marc at a level of 15% of the diet (DM basis) did not negatively affect intake. However, it has to be noted that the cows were in a low input system, with a moderate production level (~ 14 l/d in average). Milk production was not influenced by inclusion of dried grape marc in the diet (13.90 l/d in control group vs. 14.07 l/d in the grape marc group), an outcome that

suggests a potential of grape marc to replace part of the classical dietary ingredients, despite its low nutritive value (table 4).

Table 4. The effect on milk yield and composition – grape marc trial

	Treatment		***SEM***	***P value***
	Control	**Camelina**		
Milk yield, l/d	13.90	14.07	*0.563*	*0.483*
% fat	3.946	3.792	*0.175*	*0.687*
% protein	3.559	3.447	*0.048*	*0.306*
% lactose	5.017	4.999	*0.038*	*0.976*

Slight decreases of milk fat, protein and lactose concentrations were recorded, but the differences were not statistically significant.

The polyunsaturated fatty acids content was increased by the inclusion of grape marc in the diet (P=0.045), particularly the linoleic acid (P=0.018). A slight increase was also observed for linolenic acid, although the total n-3 PUFA was not significantly affected. The saturated fatty acids slightly decreased, based on the decrease of palmitic acid whereas stearic acid, of the same category, has increased. The latter is not considered to be a negative effect, as the stearic acids are regarded as having a rather neutral activity in terms of affecting consumers' health, comparing to other saturated fatty acids.

5 Conclusions and recommendations

Although the feeding trials presented herein were not conducted under controlled conditions, they provide a first estimation of the effects of Camelina meal or grape marc on milk production and milk quality. The trials were conducted in a farmer-friendly manner and the outcomes can be understood by the farmers applying similar milk production systems. The results show that these feedstuffs have a potential to be used in dairy cow nutrition. It is important to note that the trials were conducted in low-input production system farms with moderate production level (~ 14 litres of milk per cow per day).

The total replacement of sunflower meal with Camelina meal, while maintaining the nutritive supply of the diets did not influenced (P>0.05) milk yield and milk primary composition (P>0.05), with the exception of the milk fat content.

The milk fat content was significantly decreased by about 15%, a result which confirms some previous findings. This outcome raises concerns in systems where milk price at the farms' gate is corrected in view of the milk fat content. Even so, the use of Camelina meal remains a viable alternative, if its price is competitive and can be rather useful in specific feeding situations. However, more studies are needed in order to identify the mechanism responsible for this decrease in fat content and eliminate obstacles in fully valorising its potential for ruminant nutrition.

The partial replacement (30%) of corn grains and barley grains with dried grape marc, while maintaining the nutritive supply of the diets did not influence ($P>0.05$) the milk yield and primary composition of milk (fat, protein and lactose content). This replacement led to a significant increase of n-6 PUFA, especially linoleic acid. Although the concentration of α-linolenic acid was not influenced significantly, a tendency was observed.

These outcomes support the notion that grape marc can be used for short term (weeks, months) replacement of cereals without noticeable adverse effects on cows' performances. On the contrary, it has positive effects on the health value of milk, by increasing the milk content in PUFA. In general, the trials have shown that both feeds have some potential to be used as replacements for energy and protein components of the diet of dairy cows, particularly under conditions of low-input systems.

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