THESIS REPORT

The benefits of amino acids, methyl donors and B vitamins supplementation through MecoVit® product on the early lactation production and health of dairy cows

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Preface

This thesis was written as part of my end-of-year internship at the Noack group in Poland. This internship is part of the Bachelor's degree "International dairy management" that I did in Dronten at the AERES university. Noack group is a company that distributes additives and other solutions for animal nutrition. I did my 4-month internship in the ruminant team. My general mission during this internship was to follow a trial of an additive in Polish commercial farms. The additive was sourced from an Italian supplier who was also involved in the trial. The target group of this trial was dairy farmers, as this product is intended for dairy cows in transition.

I would like to thank Jan van Beekhuizen, my university coach, who answered my questions during the internship, especially concerning the writing of this thesis.

I want to thank my company coach Jedrzej Staniszkis who trusted me to give me this internship and to integrate me in the ruminant team, which I also would like to warmly thank for their experience sharing and their permanent good mood. So thank you to Patrycja, Robert and Rafal who also allowed me to visit many farms and to discover dairy farming in Poland.

A big thank you also to Jean-Loïc Martin and Richard Paratte who helped me a lot in the coordination and the data management of this trial. Thanks also to Victor Sainz de la Maza and Evert Los for their statistical advice.

Finally, many thanks to all the colleagues of Noack group who were very welcoming and kind, as well as the farmers I met.

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Abbreviations

Avg : average

BHBA : Beta-Hydroxy Butyric Acid

/d : per day

Ctrl : Control

DIM: Days in milk

DMI : Dry Matter Intake

Exp: Experimental

g : grams

Int. : Intermediate

L : Litre

mg : milligrams

Nb: number

NEB : Negative Energy Balance

NEFA : Non-Esterified Fatty Acids

SCC : Somatic Cell Count

TMR : Total Mixed Ration

VLDL : Very Low-Density Lipoproteins

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Summary

The transition period in the dairy cow should be closely monitored by farmers as it has a major influence on future lactation. The dairy cow faces a negative energy balance just after calving as she needs more calories than she takes in from her diet. Supplementation with amino acids and methyl donors can help to compensate for this and improve the beginning of lactation. The product MecoVit®, made up of choline, methionine, betaine and B vitamin, is a solution proposed by the Italian company Vetagro.

A trial was therefore conducted by Noack group, additive distributor, on 18 Polish commercial farms to determine the influence of MecoVit® supplementation on the performance and health of dairy cows in early lactation (before 60 days of lactation). Data from the 2 first post-calving milk recordings were studied and the criteria studied were: milk yield, fat percentage, protein percentage, casein percentage, lactose percentage, somatic cell count, urea and fat/protein ration. These criteria were compared between the different groups: control group (cows not supplemented with MecoVit®), experimental group (cows supplemented with MecoVit® at the recommended dose and period), intermediate A group (cows supplemented with MecoVit® during part of the early lactation period), intermediate B group (cows supplemented with MecoVit® during part of the dry period and the whole early lactation period). In this article, the results of the 3 farms that finished their trial period are presented (the results of the other farms are not usable now because they did not finish the trial period yet). On these 3 farms, 2 different dosages were tested: 50g/cow/day during the close up and fresh period or 30g/cow/day during the entire dry period and lactation. The aim of this report is to answer the following question: How does the supplementation of MecoVit® product influences the early lactation milk production and health of dairy cows in Poland?

Results highlight the following trends in the MecoVit® supplemented groups: higher milk yield; slightly lower fat and protein percentages but higher fat and protein in terms of production per day; slightly lower casein percentage; slightly higher or the same lactose percentage as the control group; lower somatic cell count, lower urea and fewer cows with a fat/protein ratio above 1.4. The differences between the groups are most of the time statistically not significant but the trends are clear. The two assays tested result in the same trends on the farms, which means that the assay not requiring close up and fresh group with a different ration can give equally good results and is therefore applicable on a larger number of Polish farms, especially small farms that do not have a different ration for close up and fresh group.

The next step is to analyse the results of all 18 farms to come to general conclusions. Another interesting trial on the MecoVit® product could be done over a longer period of time and on reproductive performance data since the MecoVit® supplementation also has the role of reducing post-calving diseases and therefore in addition to increasing production performance, also increases reproductive performance.

Chapter 1: Introduction

A. Context of the research topic

On dairy farms, the transition period of cows is a key period because it will influence the future lactation by influencing health, production, fertility and longevity. This transition period extends from 3 weeks before calving to 3 weeks after calving. The period before calving represents the close up period and the period after calving is named the fresh period. Just after the calving, dairy cows face a negative energy balance (NEB) which occurs when the cow is excreting more calories in milk than consumed by feed. Indeed, during the pre-partum period nutrients are required for foetal growth, colostrum production and finally milk production. As a consequence of NEB, body fat reserves are mobilised and used for milk production resulting in a loss of body weight. Therefore, the goal must be to maximise feed intake so that body fat mobilisation can be minimised and NEB period can be reduced. A shorter and less severe NEB may increase the early lactation performances and decrease the post calving diseases prevalence. Indeed, Goto *et al.* (2019) study proved that within 60 days after calving, 42% of cows (on the 6545 studied) present diseases. These diseases were perinatal (34%), udder (18%) or metabolic (17%) diseases (Goto *et al.*, 2019).

To prevent these diseases, the quantity of feed intake must be well monitored, but also the intake of essential amino acids and methyl donors has key role during this period. These elements can be found in feedstuff. For instance, choline can be found in soybean meal, fish meal, cottonseed meal and barley. However, results of trials highlighted that incorporate feedstuffs rich in choline in the diet of the cows can only marginally increase the post ruminal amount of choline. This low increase of post ruminal choline is due to a complete or partially degradation by the rumen microbes before that this choline can reaches the intestine. To avoid this rumen degradation as much possible, it is better to give rumen-protected choline because a bigger amount of choline will reach the intestine (Jayaprakash *et al.*, 2016). Therefore, a more efficient solution is to give feed additive for a precise supplementation and at the right time. An example of this kind of feed additive product is “MecoVit®” produced by Vetagro company and distributed by Noack Group (Table 1).

Table 1 : Composition of MecoVit® product from VetAgro company

Mecovit® composition per Kg of DM:

DL- Methionine	220 g/Kg
Choline Chloride	100 g/Kg
Betaine	30 g/Kg
BHT	2 g/Kg
Vitamin B2	0.96 g/Kg
Vitamin B12	0.014 g/Kg

It is mainly composed of methionine and choline, methyl donors that have key role in metabolism and especially during the transition period. Choline is a key compound for the synthesis of phosphatidylcholine and acetylcholine as shown in the diagram (Figure 1) (Santos & Lima, s.d.).

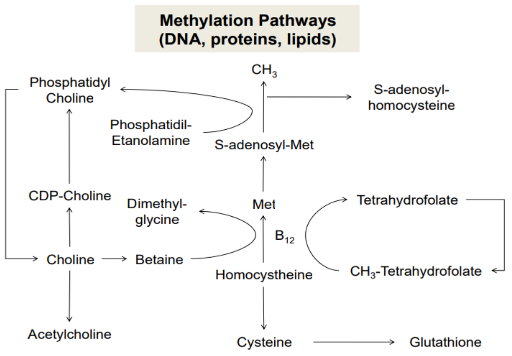


Figure 1: Metabolic pathways for choline and its relationships with folic acid and methionine as CH_3 carrier for methylation pathways

Acetylcholine is a neurotransmitter in the nervous system and useful in neuromuscular junctions for muscle contractions. Phosphatidylcholine is the major phospholipid in ruminants and it plays a role in various functions such as lipid absorption and transport, cell membrane structure, cell signalling and synthesis of lipoproteins. The phosphatidylcholine has a main role during transition period because it is the major phospholipid component of very low-density lipoproteins (VLDL). It was proven by Yao and Vance (1990) with an experiments on rats, that the lack of phosphatidylcholine synthesis due to choline deprivation leads to a decrease in VLDL synthesis, which means that choline is required for VLDL synthesis. VLDL are responsible for the export of triacylglycerol from hepatocytes. Indeed, when the NEB occurs, the cow mobilizes stored body lipids but it leads to a risk of fat accumulation in the liver. This possible fat accumulation is due to a rates of fatty acids taken up by hepatocytes higher than the possible oxidation in the mitochondria and exported as VLDL. Thus, by helping VLDL synthesis, choline supplementation can minimize the risk of fatty liver (Gruffat *et al.*, 1996) (Santos & Lima, s.d.). Moreover, carbohydrate metabolism in the liver is improved by choline supplementation because choline increases mRNA for GLUT2 which is a hepatic protein that allows glucose release from the liver into blood (Santos & Lima, s.d.).

Besides, choline is linked to other main elements for metabolism: choline is a direct precursor to betaine in methyl metabolism and choline is involved in transmethylation reaction such as methionine and carnitine synthesis and DNA methylation (Shahsavari *et al.*, 2016).

However, the effect of choline also depends on the supply of methyl donors like methionine or other cofactors associated with methylation such as B vitamins. Indeed, methionine allows choline to be more efficient by giving methyl groups. Methionine also has a supplementary role because it is a precursor of cysteine which itself acts in the synthesis of antioxidants like glutathione.

The third component of MecoVit® product is betaine which acts as a methyl donor and allows to save choline due to its high efficiency of methylation. Concerning vitamins, the vitamin B12 for instance stimulates the propionate to enter into Krebs cycle which allows to improve the production of glucose trying to reduce the NEB as much as possible (Kennedy *et al.*, 1994).

The benefits of choline, methionine and betaine have been studied in different scientific trials. The effects on feed intake, milk yield, milk quality but also on cows' health have been studied.

Concerning feed intake, results from different trials highlight that choline supplementation during the transition period allows to increase the dry matter intake (DMI) (Shahsavari *et al.*, 2016). One trial found that in the prepartum period the DMI of cows receiving choline supplementation is 12.1kg/d against 11.9kg/d for the control group and during the postpartum period it is 19.7kg/d against 19.2kg/d (Arshad *et al.*, 2020). Regarding methionine, some studies found that methionine supplementation increase the feed intake of cows before calving (Strzetelski *et al.*, 2009), or both before and after (14.79kg/d against 14.70kg/d for the control group for the close up period and 21.89kg/d against 21.17kg/d for the control group during lactation period, knowing that the supplementation in methionine was 42g/day) (Cetin *et al.*, 2018). The Monteiro et al. (2016) trial found that betaine supplementation does not change the feed intake. In this trial, cows were fed with a common diet and were supplemented with molasses-based liquid supplements made from sugar cane for the control group or from sugar cane and condensed beet soluble for the tested group (Monteiro *et al.*, 2016).

Looking at the effect on milk yield, trials highlight that methionine supplementation increase (Cetin *et al.*, 2018) (Vailati-Riboni *et al.*, 2016) or do not change the milk yield (Strzetelski *et al.*, 2009). For choline supplementation, trials show that it leads to an increase of milk production (Erdman & Sharma, 1991), which can be around 7% more compared to the control group (Santos & Lima, s.d.) whereas other found that milk yield can be a bit lower (Cetin *et al.*, 2018). Betaine supplementation from molasses-based liquid supplements in this trial, allows a better milk yield (45.1 kg/d vs. 41.9 kg/d) (Monteiro *et al.*, 2016). It seems that supplementation in choline, methionine and betaine can increase the milk yield because of a feed intake effect but also a better diet utilization effect. As an example, Monteiro et al. (2016) showed that betaine supplementation allows an increase in milk production without increasing the feeding intake, which means that the milk yield increase is due to better diet utilization.

The milk quality is also affected by the supplementation in these different elements but the results are different. Indeed, in some trials choline supplementation leads to an increase of fat content (Santos & Lima, s.d.) whereas in other trials it does not change fat content (Erdman & Sharma, 1991) or it even decrease it (Cetin *et al.*, 2018). It is the same for methionine supplementation, some found that it leads to higher fat content (Cetin *et al.*, 2018) (Osorio Estevez, 2014) and other that it does not affect this criteria (Strzetelski *et al.*, 2009). Concerning betaine supplementation, it allows higher fat content (4.78% vs. 4.34%) (Monteiro *et al.*, 2016). It is the same for protein content which seem increased by methionine supplementation (Vailati-Riboni *et al.*, 2016) (Cetin *et al.*, 2018) (Osorio Estevez, 2014) or not changed (Strzetelski *et al.*, 2009) and not changed (Erdman & Sharma, 1991) or decreased (Cetin *et al.*, 2018) by choline supplementation.

Moreover, another trial highlights that methionine, choline and betaine supplementations have complementary effects because the choline supplementation allows the best milk yield, methionine supplementation allows the best protein content and betaine supplementation allows the best fat content (Table 2) (Davidson *et al.*, 2008).

Table 2: Daily milk yield composition, intake, BW, and BCS as affected by dietary treatment for multiparous cows (n=12 per treatment) (Davidson et al., 2008)

Item	Dietary treatment[1]				SEM	$P \leq$
	Control	RP-MET	RP-BET	RP-CHOL		
Milk						
Yield, kg/d	37.7^{b}	39.8^{b}	38.6^{b}	44.1^{a}	1.2	0.01
True protein, %	2.47	2.61	2.44	2.49	0.06	0.18
True protein, kg/d	0.92^{c}	1.04^{ab}	0.96^{bc}	1.10^{a}	0.04	0.01
Fat, %	2.80	2.46	2.91	2.64	0.13	0.11
Fat, kg/d	1.03^{ab}	0.99^{b}	1.11^{ab}	1.16^{a}	0.05	0.05
MUN, mg/dL	17.0	16.0	17.4	17.2	0.8	0.58
DMI, kg/d	21.9	20.8	21.7	24.3	1.4	0.32
ECM,[2] kg/d	32.5^{b}	33.8^{b}	34.3^{b}	37.9^{a}	1.1	0.01
3.5% FCM,[3] kg/d	32.8^{b}	33.3^{b}	34.9^{b}	38.0^{a}	1.1	0.01
ECM/DMI, kg/kg	1.56	1.80	1.67	1.68	0.15	0.73
BW, kg	574	606	577	607	15	0.26
BCS[4]	2.25	2.32	2.17	2.14	0.10	0.59

[a–c]Means within a row lacking a common superscript differ ($P < 0.05$).
[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.
[2]ECM (kg/d) = [milk yield (kg/d) × 0.327] + [milk fat (kg/d) × 12.86] + [milk true protein (kg/d) × 7.65] (Dairy Records Management Systems, 2006).
[3]3.5% FCM (kg/d) = [milk yield (kg/d) × 0.4324] + [milk fat (kg/d) × 16.2162] (Dairy Records Management Systems, 2006).
[4]5-point scale where 1 = very thin to 5 = obese (Ferguson et al., 1994).

Also, Ardalan et al. (2010) studied choline and methionine supplementation but also the effect of a mixed supplementation. They concluded that the effects on milk yield, milk fat and milk protein were more pronounced at the mixed supplementation (Table 3).

Table 3: The effects of supplemental rumen-protected methionine and choline on productive indices of lactating dairy cows across the lactation weeks (Ardalan et al., 2010)

Item	Treatment				SEM	p-Value			
	Control	RPM	RPC	RPM + RPC		Trt	Parity	Time	Trt × time
Milk, kg/day	30.71^{c}	32.64^{bc}	34.23^{ab}	35.14^{a}	0.97	0.014	<0.001	<0.001	0.999
4% FCM, kg/day	26.55^{c}	28.59^{bc}	30.37^{ab}	31.40^{a}	1.06	0.003	<0.001	0.016	0.943
ECM, kg/day	29.28^{c}	31.47^{bc}	33.21^{ab}	34.34^{a}	1.10	0.004	<0.001	0.001	0.983
Fat, %	3.29	3.30	3.28	3.39	0.03	0.904	0.006	<0.001	0.900
Protein, %	3.10	3.16	3.09	3.12	0.02	0.364	0.275	<0.001	0.777

Means with different superscripts at each row differ significantly ($p < 0.05$). Trt, treatment; RPM, rumen-protected methionine; RPC, rumen-protected choline.

Beyond the effect on milk parameters, choline, methionine and betaine supplementation has effects on cows' health. Several trials concluded that choline supplementation leads to a decrease of non-esterified fatty acids (NEFA) concentration in blood (Koujalagi *et al.*, 2018) (NEFA; 703 Eq/L vs. 562 Eq/L) (Cooke *et al.*, 2007). Moreover, thanks to choline, the β-hydroxy butyric acid (BHBA) level declines and the mean lipid per oxidation level increases (Koujalagi *et al.*, 2018). In addition, the plasma concentration of glucose tended to be increased by choline supplementation of 20g/d during 7 days before calving and 21 days after calving (Xu *et al.*, 2006).

In addition to these modifications of blood parameters concentration, it was proven that choline supplementation reduces the prevalence of various diseases. First, choline supplementation prevent clinical and subclinical ketosis but also relapse of ketosis (Table 4) (Santos & Lima, s.d.).

Table 4: Effect of feeding rumen-protected choline (RPC) on ketosis in dairy cows (Santos & Lima, s.d.)

	Treatment		P
	Control	RPC	
	---------------- % (no./no.) --------------		
Ketonuria[1]	28.8 (51/177)	10.7 (19/177)	0.001
Clinical ketosis[2]	11.2 (20/177)	4.0 (7/177)	0.01
Relapse of ketosis[3]	6.8 (12/177)	2.3 (4/176)	0.05
Subclinical ketosis[4]			
1 d postpartum	37.2 (68/183)	28.5 (51/179)	0.07
14 d postpartum	29.7 (52/175)	19.9 (35/176)	0.35

[1] Ketonuria using Ketostix reagent strips (Bayer Diagnostics).
[2] Anorexia, depressed attitude, severe ketonuria using Ketostix, including some cases of nervous ketosis.
[3] Proportion of all cows with more than one case of clinical ketosis.
[4] Subclinical ketosis = plasma 3-OH-butyrate > 1,000 µMol/L.

The choline allows to increase the expression of fatty acids transport protein and carnitine transporter. Because these proteins are involved in fatty acids uptake and intracellular transport in the liver, they can allow to reduce the triacyl glycerides accumulation in the liver, so prevent fatty liver, by stimulating the fatty acids oxidation (Shahsavari *et al.*, 2016).

In addition do that, choline supplementation during transition period prevents other diseases like metritis, displaced abomasum, mastitis (Table 5) (Santos & Lima, s.d.) but also lower number of stillbirths (Shahsavari *et al.*, 2016).

Table 5: Effect of feeding rumen-protected choline (RPC) on dairy cows' health (Santos & Lima, s.d.)

	Treatment		P
	Control	RPC	
	----------- % (no./no.) -------------		
Retained placenta	11.1 (20/179)	10.1 (18/178)	0.72
Postpartum fever	31.1 (55/177)	31.6 (56/177)	0.77
Puerperal metritis	3.4 (6/177)	3.4 (6/177)	0.69
Metritis	11.3 (20/177)	7.9 (14/177)	0.33
Displacement of abomasum	4.5 (8/177)	2.3 (4/177)	0.77
Mastitis	22.5 (39/173)	14.7 (26/176)	0.06
Postpartum disease[1]	57.1 (101/177)	38.4 (68/177)	0.001
Death	7.1 (13/183)	4.5 (8/179)	0.27
Left study[2]	9.8 (18/183)	7.8 (14/179)	0.48

[1] Includes retained placenta, metritis, clinical ketosis, displacement of abomasum, and mastitis.
[2] Cows leaving the study prior to 80 days postpartum because of death or culling.

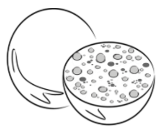
Methionine supplementation also has a positive effect on cows' health because it allows a faster recovery to a positive energy balance and a lower risk to develop ketosis (Osorio Estevez, 2014). Moreover, methionine supplementation enables better immune and antioxidant status of cows, which are key parameter around calving (Vailati-Riboni *et al.*, 2016). The antioxidant status is indicated by total antioxidant capacity, glutathione peroxidase activity and vitamin E concentration (Sun *et al.*, 2016). Following this better status, it allows better reproductive performances such as reduced open days and number of inseminations (Table 6) (Ardalan *et al.*, 2010).

Table 6: The effects of supplemental methionine and choline on reproductive indices of Holstein dairy cows (Ardalan et al., 2010)

	Treatment					p-Value		
Item	Control	RPM	RPC	RPM + RPC	SEM	Trt	Parity	Trt×Parity
Days to first oestrus	61.8	48.8	64	31.6	7.4	0.325	0.08	0.364
Open days	115.4[a]	88.6[ab]	106.6[ab]	73.8[b]	9.3	0.002	0.663	0.739
Services per conception	3[b]	2.2[ab]	2.4[ab]	2[a]	0.2	0.034	0.096	0.425
Number of pregnant cows	3	6	6	8	0.6	0.064	0.125	0.534

Means with different superscripts differ significantly ($p < 0.05$). Trt, treatment; RPM, rumen-protected methionine; RPC, rumen-protected choline, SEM, standard error of means.

Because of all these benefits of choline, methionine and betaine supplementation, the product proposed by Vetagro company is a combination of this beneficial elements. The speciality of this product lies also in the fact that it is microencapsulated. Free amino acids are mainly degraded in the rumen. To be available and absorbable in the duodenum for metabolism, they must be administered in a protected form. Paratte et al. (2013) study tested the release of methionine, lysine and proline, by an in vitro incubation process with ruminal juice. The amino acids were protected by a hydrogenated vegetable fat matrix (patented technology). A small amount of each protected amino acids was incubated with Van Soest buffer (40 ml) and rumen juice (10 ml) taken from 2 cows within a maximum of 24 h. Samples were taken at 0, 4, 8, 16 and 24 hours for the determination of amino acids protection and release rates. The methionine residue (%N) was found to be above 80% up to 8 hours of incubation and then decreased to 62% and 47% at 16 and 24 hours respectively. These high release rates are allowed by the microencapsulation of the amino acids (Paratte *et al.*, 2013). There are more different types of microencapsulation. The 2 most common are the lipid matrix type and the shell type (Figure 2).



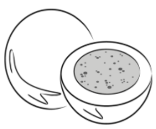


Figure 2 : Microencapsulation types : matrix type (up) shell type (below) (Vetagro, 2020)

The matrix type is less sensitive to external factors because of its flexibility, opposite to the rigidity of the shell type. With the matrix type, the bypass effect and slow release capacity is maintained even if the product is cracked, scratched, peeled, size-reduced or melted. Vetagro's technology ensures that the nutrients are absorbed throughout the intestinal tract because amino acids stay protected in the rumen and the lipid matrix start to be digested by lipases in the intestine, which make the amino acids available to be absorbed (Vetagro, 2019).

MecoVit® is a microencapsulated product, so the different components (methionine, choline, betaine, B vitamins) are protected with the matrix type of microencapsulation. Another product from Vetagro company is encapsulated in the same way but it contains only one of the components of MecoVit® which is the methionine. This methionine microencapsulated product is called Timet®. Trials on Timet®

product have already been made to prove the efficiency of this mode of encapsulation and the efficiency of methionine. Trials highlight that microencapsulated methionine supplementation leads to higher milk yield (Abedal-Majed *et al.*, 2021) (Table 7) (Vetagro, 2019).

Table 7: Performance of 48 Holstein dairy cows (with an average of 100 days in milk) consuming Timet during 28 days (Vetagro, 2019)

	Dietary Treatment[1]				SEM[2]	*P*-value[3]			
	CTR	T20	T35	T50		Trt	Lin	Quad	Cubic
Dry Matter Intake, kg/day	23.59	23.39	23.02	23.33	0.46	0.64	0.39	0.49	0.48
Milk Yield, kg/day	38.05	39.31	39.16	39.73	0.87	0.11	0.03	0.53	0.41
3.5% FCM[4]	37.77[b]	40.88[a]	40.15[a]	40.90[a]	1.08	0.01	0.007	0.13	0.17
ECM[5]	38.35[b]	41.45[a]	40.90[a]	41.67[a]	1.05	0.009	0.004	0.14	0.21
Protein, %	3.17	3.16	3.22	3.21	0.04	0.18	0.11	0.66	0.15
Protein, kg	1.16[b]	1.24[a]	1.25[a]	1.27[a]	0.03	0.003	0.0006	0.23	0.36
Fat, %	3.72	3.69	3.70	3.66	0.88	0.83	0.42	0.87	0.67
Fat, kg	1.35[b]	1.45[a]	1.43[a]	1.45[a]	0.05	0.03	0.02	0.14	0.25
Lactose, %	4.66	4.63	4.67	4.62	0.03	0.35	0.37	0.69	0.13
Lactose, kg	1.71[b]	1.85[a]	1.82[a]	1.86[a]	0.05	0.02	0.009	0.17	0.24
MUN, mg/dL	13.58	13.55	13.71	13.32	0.23	0.58	0.41	0.38	0.49
Somatic Cell Count	281.42	394.01	222.94	388.52	149.48	0.58	0.73	0.87	0.18
Body Weight, kg	672.82	671.48	670.03	671.54	8.51	0.82	0.54	0.53	0.69
Body Condition Score[6]	3.34	3.33	3.32	3.32	0.03	0.83	0.40	0.69	0.99

[1]**CTR** = Control; **T20** = CTR + Timet® 20 g; **T35** = CTR + Timet® 35 g; **T50** = CTR + Timet® 50 g
[2]Standard error of treatment mean is shown.
[3]Main effect of treatment.
[4]3.5% Fat corrected milk = [milk fat (kg) × 16.216] + [milk yield (kg) × 0.4324].
[5]ECM = [0.327 × milk yield (kg)] + [12.95 × milk fat (kg)] + [7.65 × milk protein (kg)].
[6]Body condition scores 1-5 scale (Wildman et al., 1982).
[a-d]Values in the same row with different superscript differ significantly.

Some trials also found that the Timet® product enables an increase of protein content (King *et al.*, 2021), others an increase of fat content (Abedal-Majed *et al.*, 2021) and other trial proved both increase (Table 8) (Sainz de la Maza *et al.*, 2019).

Table 8 : Mean values +/- standard deviation of protein, fat, urea (Sainz de la Maza et al., 2019)

Item	CTR1	TMT	CTR2	*P*-value
Protein (%w/w)	3.35 ± 0.021[b]	3.43 ± 0.020[a]	3.38 ± 0.026[ab]	0.026
Fat (%w/w)	3.63 ± 0.027[b]	3.75 ± 0.026[a]	3.71 ± 0.034[ab]	0.013
Urea (mg/L)	193.0 ± 6.89[a]	180.8 ± 6.64[a]	189.9 ± 8.78[a]	0.434

Moreover, Timet® supplementation allows supplementary benefits such as a lower urea rate in milk (180.8 mg/L compared with CTR1 193.0 mg/L and CRT2 189.9 mg/L) (Sainz de la Maza *et al.*, 2019) but also a lower urea rate in plasma, which shows a more efficient utilization of dietary nitrogen (Gallo *et al.*, 2010). Indeed, methionine supplementation improves the nitrogen efficiency by allowing more protein accretion in many tissues.

Trials have also been done on MecoVit® product. Results of a trial show that milk quantity and quality are improved because according to the table 9, results present higher milk yield and fat content in the experimental group compared to the control group (Shepelev *et al.*, 2021). In this trial, the difference between the ration in these 2 groups was only the supplementation of 50g of methionine per cow and per day during 40 days. However, such a huge difference in milk yield can not only be explained by this methionine supplementation so it can also be due individual milk production capacity of cows selected and a bit higher parity in the experimental group than in the control group (2.4 vs 2.3).

Table 9 : Yield and fat content of milk produced by cows supplemented in MecoVit® or not (Shepelev et al., 2021)

Lactation month	**Milk yield average (kg/head/day)**	**Milk fat content (%)**	**Milk yield average (kg/head/day)**	**Milk fat content (%)**
	Control group		Experimental group	
1	35.1	3.64	35.9	3.72
2	34.8	3.68	41.0	3.72
3	34.3	3.72	38.8	3.74
4	31.6	3.76	34.7	3.76
5	29.5	3.78	34.5	3.78
6	28.2	3.81	33.2	3.81

MecoVit® supplementation also lowers the circulating fatty acids in postpartum dairy cows and thus slows the progression of fatty liver disease in these cows (Fontoura *et al.*, 2019). Indeed, the increased in hepatic lipid content (from day 5 to 14 after calving) is supressed with MecoVit® supplementation relative to control (3.57 vs. −0.29%) (Zang *et al.*, 2019).

B. Elaboration of the theorical framework and knowledge gap

During the trials described above, of MecoVit® product was offered 50 g/head/ day during a period of 20 days before and 20 days after calving (Shepelev *et al.*, 2021). This dosage corresponds to the recommendations for the standard dosage given to the close up group and the fresh group (Figure 3).

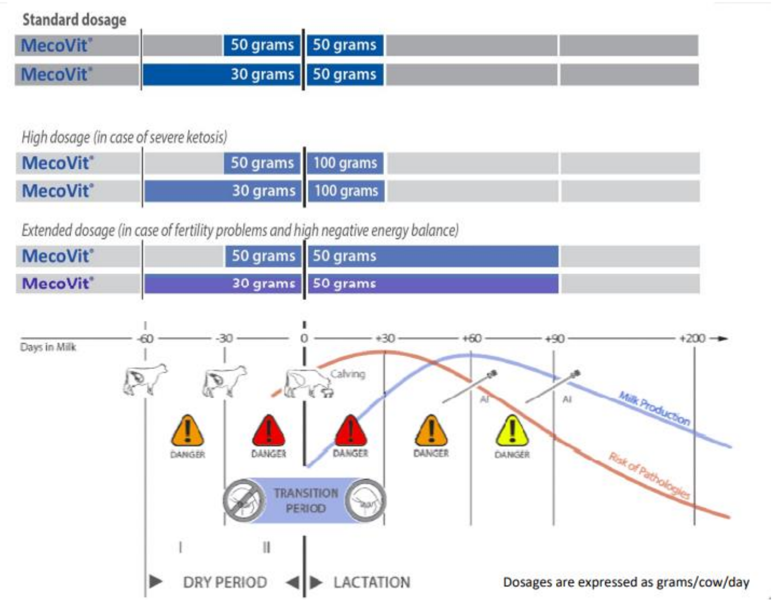


Figure 3 : MecoVit® dosage recommendations (Vetagro, 2020)

However, the efficiency of MecoVit® product with other amount and time of supplementation is not known. There is a particular interest in the Polish market for these kind of products, however most dairy farms in Poland are small (76% of dairy farms have less than 50 cows (Figure 4) (pfhbipm, 2021)) and they do not split their cows in a close up group and postpartum group. These farms have only a group of dry cows and a group of lactating cows, which makes impossible to give MecoVit® only during the transition period. Another option also possible on polish farms is to split the lactating cows in 2 with a group for the first 180-200 days of lactation and the second group for the following time of lactation. To summarize the situation on polish farms, these 3 possibilities are possible:

- 1 diet for dry cows and 1 diet for lactating cows
- 2 diets for dry cows (including close up diet: 21 days before calving) and several diets for lactating cows (including fresh diet: 21 days after calving)
- 1 diet for dry cows and 2 diets for lactating cows (including 1 diet for the first 180-200 days of lactation)

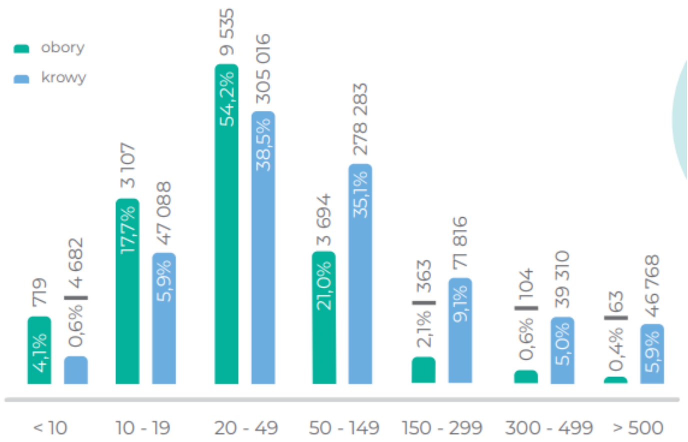


Figure 4 : Repartition of the number of cows (blue) and number of farms (green) according to the farm size (in number of cows) (pfhbipm, 2021)

Dairy cows are mainly located in the north-west part of the country, in Grater Poland, Podlaskie, Masovia, Warma-Masuria and Kuyavia-Pomerania regions (Figure 5). Dairy farms in these regions are rather small, with an average number of cows between 39.7 and 49.7 (Figure 6)

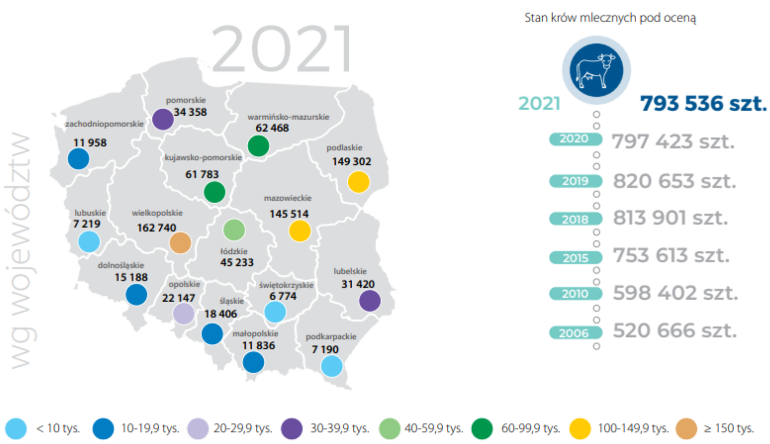


Figure 5 : Number of dairy cows in each region of Poland

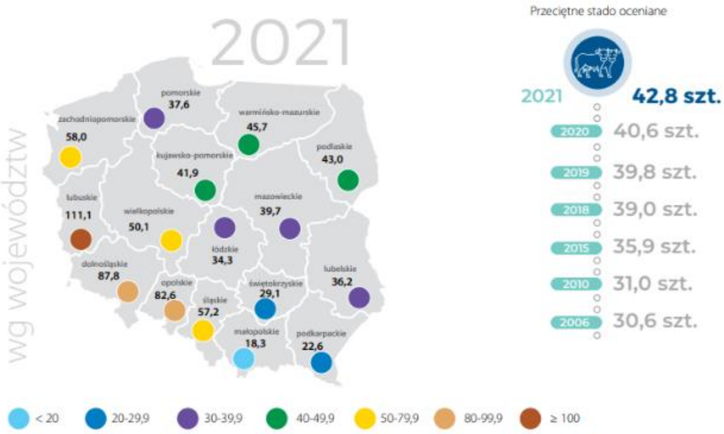


Figure 6 : Size of the dairy farms (in number of cows) in each region of Poland (pfhbipm, 2021)

Moreover, it is also in these regions that the milk yield and milk protein and fat content are rather low compared to other regions (Figure 7-8). These lower milk quantity and quality can be explained by a very simple feed management with very variable forage quality and not necessarily a search for improvement or use of additives on these farms with low animal numbers. It means that there is room for improvement in these regions and MecoVit® product can help to increase these parameters. The interval between calving is also high compared to other regions because it is between 421 and 435 days (Figure 9).

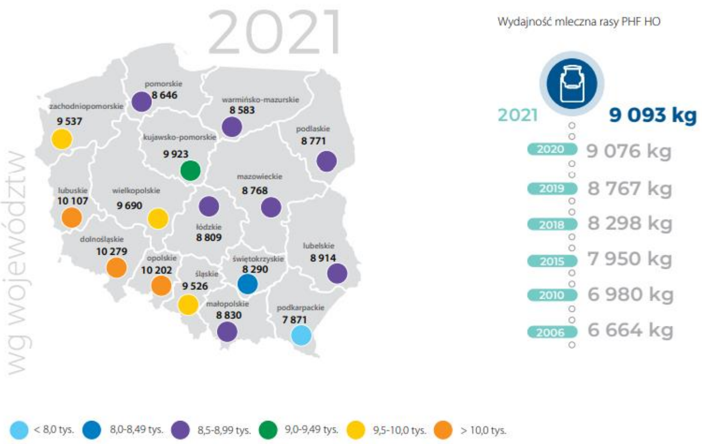


Figure 7 : Average milk yield in each region of Poland (pfhbipm, 2021)

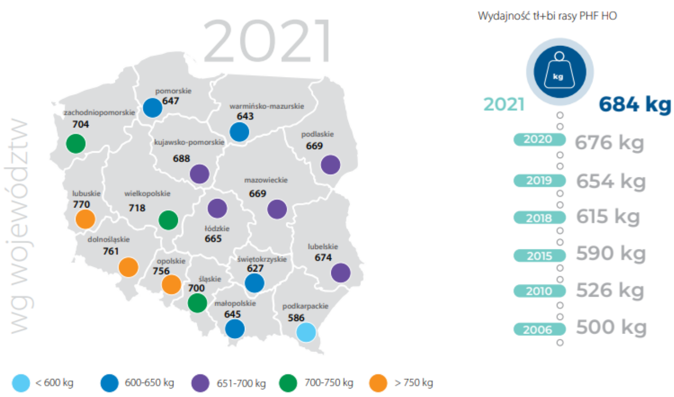


Figure 8 : Average fat and protein content of milk in each region of Poland (pfhbipm, 2021)

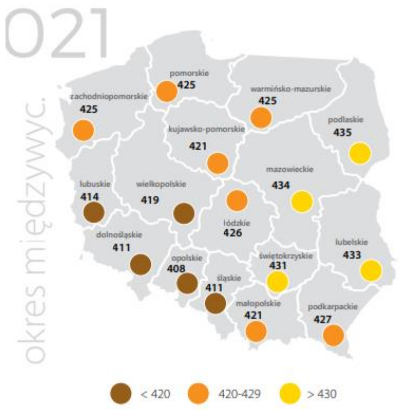


Figure 9 : Calving interval in each region of Poland (pfhbipm, 2021)

Because MecoVit® product can improve cows health just after calving, the use of this product can be a mean of improvement for the success of insemination which can lead to a better calving interval. For instance, avoid ketosis issue can allow better reproduction performances and according to Gulinski (2019) trials made from 2014 to 2017 on 10 herds located in easter Poland, the occurrence of subclinical ketosis is 19.9% and 3.3% for clinical ketosis. On this trial, it was considered that the fat and protein ratio between 1.41–1.7, indicating subclinical ketosis and this ratio above 1.7, indicating clinical ketosis (Gulinski, 2019). Another trial carried out on different European countries showed that the odds of sub clinical ketosis were twice as likely in Poland compared to Austria (Bjornsdotter Berge, 2020).

Thus, these farms without proper close up and fresh group represent a huge part of the polish dairy farms. It means that on these farms, MecoVit® cannot be given only during the period of 20 days before and 20 days after calving because there is no proper diets for this period. Therefore, a solution can be to give MecoVit® during a longer time, that is to say during all the dry period and during the first 180-200 days in lactation or even during all the lactation. This new period of giving MecoVit® leads to a change in the amount of MecoVit® given from 50g to 30g per cow and per day (see experimental design). The predominance of small farms in Poland justifies the interest to study the use of MecoVit® with a period and amount adapted to these farms. Especially because the quantity and quality of milk produced can be improved on these farms, as can the prevalence of ketosis and reproductive performances. Moreover, improving productive and reproductive performances leads to a reduced environmental impact of milk production which is also an important challenge in the current context of climate change.

C. Main question and sub-questions

According to this knowledge gap, the main question is: How does the supplementation of MecoVit® product influences the early lactation milk production and health of dairy cows in Poland?

Answering these main questions requires to answer to the following sub questions:

- How are different dosage and time of giving of MecoVit® impacting the milk yield in early lactation in Poland (<60 days of lactation)?
- How are different dosage and time of giving of MecoVit® impacting the milk quality parameters in early lactation in Poland (<60 days of lactation)?
- How are different dosage and time of giving of MecoVit® impacting the cow's health and particularly the risk of ketosis in early lactation in Poland (<60 days of lactation)?

D. Objectives

The objective of this trial was to evaluate and demonstrate the effects of MecoVit® supplementation (with a dosage and time adapted to farm management) in commercial polish dairy farms. This trial studied the effects on milk quantity and quality and the health of dairy cows in early lactation. These results will allow to companies manufacturing (Vetagro) and distributing (Noack group) this product to prove its efficiency to their current and potential clients. The information about this trial could be disseminated through leaflet, articles, presentations to the farmers. These outcomes presented to the target group will allow them to take the decision of starting to use this MecoVit® product on their farm.

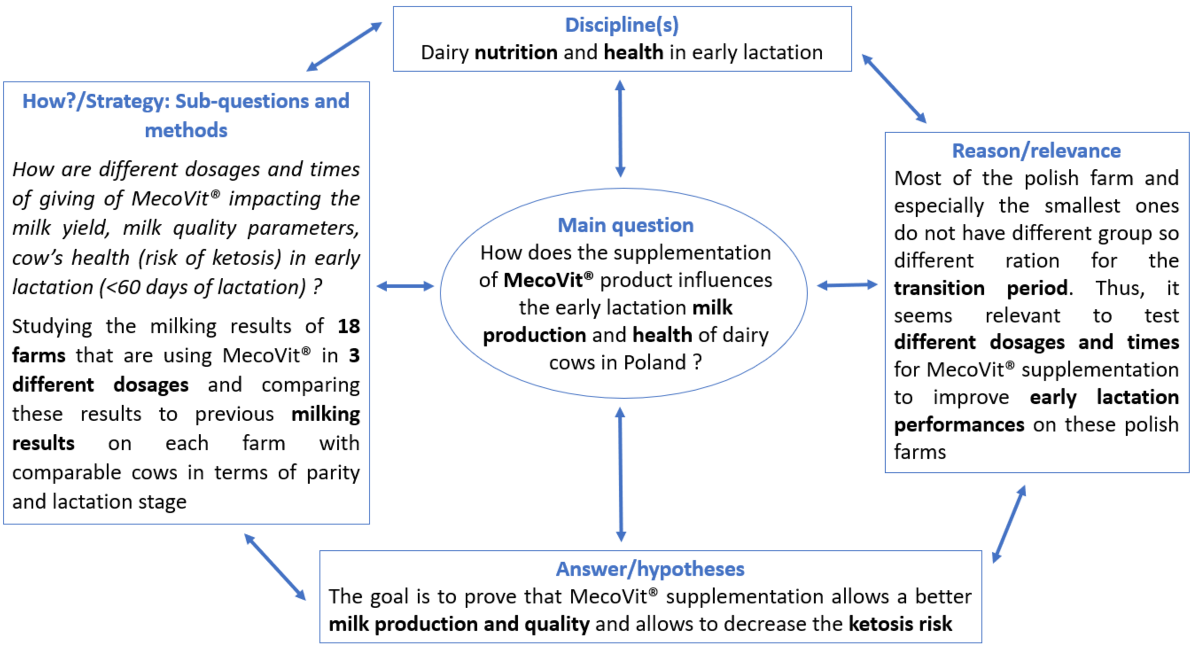


Figure 10: Summary diagram of the research proposal

Chapter 2: Materials and methods

A. Materials

a. Dairy farm selection, description and requirements

For this trial, Noack ruminants team was involved in collecting the data and analyzing the data under supervision of Tamas Kertesz and Richard Paratte from Vetagro. This MecoVit® trial was run in 18 farms located mostly in the north-east of Poland (Figure 11).

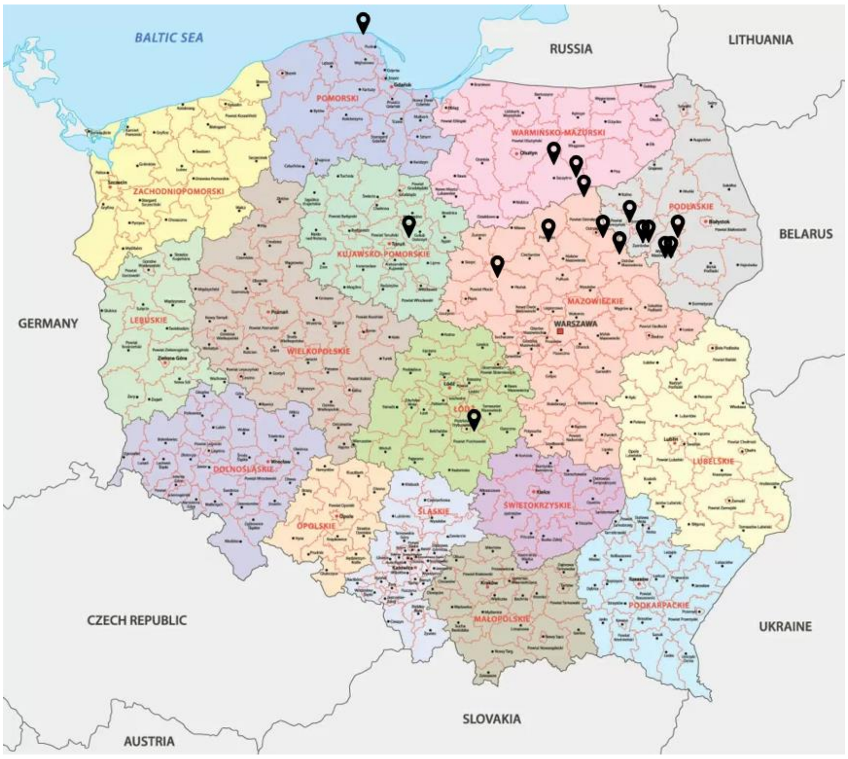


Figure 11: Localisation of the farms involved in the MecoVit® trial

On these farms, the average number of cows was 99 for the total and 87 for the lactating cows. The smaller farm owns 25 lactating cows and the biggest one 338 lactating cows. Farmers were contacted by regional managers from cattle department during their meetings because they propose them to use MecoVit® when it seems to be a good solution on their farm. Then, when farmers accepted to order MecoVit® and to use it before May, then they were part of the trial if they accepted.

The necessary conditions that need to be fulfill by the farms to be part of the trial were the following ones:

- Availability of milk recordings: monthly recordings for the herd and individual recordings from the polish federation of cattle breeders and milk producers
- Availability of these milk recordings for 2 months previous the trial and for the duration of the trial
- Supplying continuously MecoVit® during the trial period

Moreover, supplementary data were required for approvement by Jean-Loïc (Ruminants technical manager from Noack) and Richard Paratte (Ruminants technical sales and support manager from Vetagro). These data were:

- General information about milk production on the farm:
  - Number of lactating cows/ total cows;
  - Average milk production and milk quality (Fat, Protein, Lactose, Urea, Somatic Cell Count)
  - Average Days In Milk
  - Average parity
  - Particular situations/problems
- Identification of all the animals involved in the trial: All cows involved in the trial has to be uniquely identified with Ear-Tag ID number in order to be able to follow them during the trial.
- Rations composition of all groups (dry period, lactating phases) comprehensive of all components including the feed mixes.
  If some treatment after calving is done (glycerol, propylene glycol,…) it has to be reported.
- Product quantity used for the trial has to be calculated and approved.

The goal of this farms selection was to have a sample of farms that are representative of polish dairy farms and with different rationing organization.

b. Experimental dairy cows used

On these 18 farms, the dairy cows were all Holstein cows. On each farm, the cows studied were those under or at exactly 60 days of lactation during the trial period of 2 or 4 months (according to the case on each farm: see experimental design part). It means that only the 2 first monthly milk recordings after calving were studied. It also means that a same cow can be present 2 times in the data file: a first time for the first milk recording (<30days) and a second time for the second milk recording (<60days). This repetition is not a problem as the results for the first and second recording were studied separately. Also, cows that are still producing colostrum were not used because the milk data were not usable. The group in which cows are allocated according to their intake of MecoVit® will be explained in statistical analysis method part.

c. **MecoVit®** product

This product from Vetagro company is a special mix of rumen-protected microencapsulated methionine, choline, betaine and B vitamins (see Table 1 at the beginning). These different nutrients are playing a crucial and synergistic role in the metabolism of the transition cow. This combination of nutrients allows to reduce liver steatosis, ketosis and incidence of postpartum diseases. Thus, the MecoVit® improves reproductive and productive efficiency of the transition cow. Moreover, the nutrients are micro-encapsulated in an exclusive and patented vegetable fat matrix which ensure an excellent by-pass of the rumen and a high availability in the intestine. The release of the nutrients starts in contact with lipase and other secretions in the intestine. In brief, the nutrients in MecoVit® have the following role:

- Choline prevents the abnormal accumulation of fat in the liver
- Methionine supports the choline action by giving methyl group (CH_3) and is a precursor of cysteine (antioxidant)
- Betaine acts as a methyl donor
- BHT (Butylated hydroxytoluene) is an antioxidant
- B vitamins allow propionate to enter the Krebs cycle to produce more glucose

B. Methods

a. Experimental design

MecoVit® dosage used on each farm was adapted according to farm situation. When there was only one total mixed ration (TMR) for the lactating cows, 30g/cow/day of MecoVit® were given to all cows in dry and lactation period. This first dosage is the case 1. The case 2 was applied on farms where there were close up group (different TMR 3 weeks before calving) and fresh group (different TMR 3 weeks after calving). In that case, 50g/cow/day of MecoVit® were added to the TMR of these 2 groups which means that MecoVit® were given during the transition period only. Finally, when there were 1 or 2 groups of lactating cows but no proper group for transition cows, the case 3 was applied: 50g/cow/day of MecoVit® for the dry cows and 30g/cow/day of MecoVit® during the first 180-200 days of lactation. MecoVit® dosages according to the cases are summarized in the table 10. Thus, the trial period (defined as the period during which MecoVit® is given) is 2 months for the case 2 (close up period of 1 month and fresh period of 1 month) and 4 months for the case 1 and 3 (dry period of 2 months + first 2 months of lactation). It was not manageable on these farms to keep a control group to which exactly the same diet would have been given except MecoVit®. Therefore, the control group used are cows from a previous period. For each farm, the milk recordings of 2 months before the start of MecoVit® were used as control. From these 2 milk recordings, the cows in their 2 first months of lactation were studied as control cows (see different groups in statistical analysis part).

Table 10: Calculation of the MecoVit® quantity required per head according to the dosage applied

		DRY PERIOD & PRECALVING			LACTATION			TOTAL/Head (kg)
		Dosage/cow/day (kg)	Days	Total/head (kg)	Dosage/cow/day (kg)	Days	Total/head (kg)	
1	Only one TMR for Lactation	0,03	60	1,8	0,03	60	1,8	3,6
2	Close up + Fresh	0,05	30	1,5	0,05	30	1,5	3
3	Dry + 1st group	0,05	60	3	0,03	60	1,8	4,8

b. Data description and collection procedure

Milk recordings were asked by cattle managers to the farmers who received it from the polish federation of cattle breeders and milk producers in PDF or Excel. Only monthly milk recordings were available because more regular controls (weekly or daily) would have required additional costs and workload for farmers which are more difficult to apply on commercial farms (as opposed to a trial on experimental farms). Individual cow performances were analyzed to study the effects of MecoVit® supplementation.

The data kept on an excel file from the milk records were:

- cow identification,
- lactation number,

- last calving date,
- milk recording date,
- days in lactation,
- milk yield (kg/day),
- percentage of fat,
- percentage of protein,
- percentage of casein,
- percentage of lactose,
- value of Somatic Cell Count (SCC),
- value of urea (mg/L)
- fat/protein ratio

Some data were added on the Excel file such as:

- category of days in lactation (<30 or <60),
- starting date of MecoVit® use,
- number of days with MecoVit® supplementation during dry/close up period and during lactation/fresh period

Moreover, if the farmer could provide it during the visit, the precise ration of all the cows group were listed and also data about dairy cows' health in early lactation, such as prevalence of post-partum diseases or fertility data that can reflect the health.

An excel file was done for each farm and the results were first analyzed per farm. This choice was made because the goal was first to present the results to the farmers on their own farm in order to show them the benefits of MecoVit® use. Then, a grouping of data from the different farms could be done, but only for those that are in the same case (same quantity and same duration). In this way, the data can be compared without forgetting to take into account the effect of the different practices on each farm and mainly the different rations. However, this grouping of farms by case was not studied in this report because too few farms had completed the trial by the end of June (time of the end of the internship). Indeed, in this report the statistics were done only of the 3 farms that had completed the trial period by the end of the placement. This choice was made because the deadline for submit the thesis report was before middle of July so the statistics was done on the available data et the end of June.

c. Statistical analysis

Early lactation performances were monitored by comparing the performances of fresh cow that received MecoVit® with fresh cows that had not received this supplementation and that are around the same lactation stage. For doing this, each cow will be assigned to one category:

- <u>Control group:</u> No MecoVit® received at all
- <u>Intermediate A group:</u> MecoVit® received during a part of the fresh/early lactation period
- <u>Intermediate B group:</u> MecoVit® received during a part of close up/dry period and received during fresh/early lactation period
- <u>Experimental group:</u> MecoVit® received during all the close up/dry period and during fresh/early lactation period

All the cows from the experimental group were kept and then for the 3 other groups, cows with the same parity were randomly chosen in each of the 3 other groups when it was possible. The goal was to have the same parity average for each group in order to have comparable data. Then analysis was done in order to compare the results of cows according to their group so according to their supplementation in MecoVit®. The data analysis was done with Excel and SPSS. The calculations were mostly calculations of the average of milk yield, fat, protein casein and lactose percentage, SCC, urea and ratio of fat and protein. These averages will be calculated for each group of cows (previously presented) on each farm and for the first and second milk recordings after calving. T-tests and Anova tests were used to study if the differences of milk parameters between the groups are significant.

C. Specific information about the 3 farms analysed

Due to the deadline of this thesis report, the results of only 3 farms were analysed in this report as explained before. The table 11 presents the general information concerning these 3 farms.

Table 11 : General information about the 3 analysed farms

	Total cows	Lactating cows	Experimental case	Start of MecoVit® use
Farm 1	395	340	Case 2	17/01 (for close up group) 25/01 (for fresh group)
Farm 2	57	50	Case 1	15/04
Farm 3	115	104	Case 2	25/03

An important criteria to take into account is the ration on these 3 farms because it also has an impact on the milk production. The different rations for dry and lactating cows from each farm are presented table 12.

Table 12: Dairy cows' ration (lactating and dry) in 3 analysed farms (all components are in kg of fresh matter/day/cow)

	Lactating cows			Dry cows		
	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
Maize silage	24	30	35.5	4		5
Alfalfa	4					
Grass silage	3	10	14	20	X*	15
Maize grain	2.5	2.7				
Soybean meal	2.5	1		0.7		0.1
Brewery grain from corn	4					
Beet Pulp		2				
Grass bales	2					
Melassa			0.3			
Barley	1.5		2			
Triticale		3.5				
Bread (waste)	1.2			0.5		
Rapeseed meal	1	2.5	2	1.3		0.6
Wheat straw	0.25		1.4	2.7	X*	2.5
Limestone	0.15	0.2	0.1			
Salt (sodium chloride)	0.04	0.07	0.1			
Magnesium oxide	0.02					
Urea		0.3	0.1			
AcidBuf		0.1	0.05			
Mlekovit extra		0.2				
Easylin 60		0.3				
Yeast		0.1				
Buffer (NaHCO3)		0.07				

*means that this element is present in the ration but the quantity was not known by the farmer

Chapter 3: Results

As a reminder, the data collected for this trial are the monthly milk recordings and, in these recordings, only the cows in their first 60 days of lactation are analysed as it is the performance of the beginning of lactation that is studied. These data contain information about the cows (parity, calving date, number of days in milk) and information about their production (milk quantity, fat, protein, lactose, casein, urea, SCC). In this section, the results of the 3 farms that completed the trial period will be presented. The farms are studied separately since different cases were used (case 1 and case 2) and a global analysis of the results will be done once all 18 farms have completed the trial period. The results are all presented in tabular form in order to be more concise as many figures are presented here. Graphs will be used more for the oral presentation part. In this section, the results will simply be presented objectively, and they will be interpreted and discussed in the following discussion section.

The 4 groups studied are Control, Experimental, Intermediate A and Intermediate B. The definition of these 4 groups was explained earlier in the materials and methods section. In table 13, the main information about each group on each farm are presented. The aim was to have groups that were as comparable as possible in terms of number of individuals, parity and days in milk. However, this was not always possible, especially on farms 2 and 3 as the number of cows was smaller. Moreover, there were sometimes no cows in the experimental group (having received the full dosage of MecoVit®) that were less than 30 DIM (Farm 2) or between 30 and 60 DIM (Farm 3) as it depends on the calving dates on the farm. This reason explains why for the farm 2 there will be only data about the second milk recording (<60d) and for the farm 3 only data for the first milk recording (<30d) that will be presented.

Also, primiparous and multiparous cows were studied separately in some tables but only on farm 1 which had a sufficient number of cows to study them separately unlike the other 2 farms where the number of animals is much more restricted.

Table 13 : Groups composition on each of the 3 farms

		< 30 days in milk					< 60 days in milk				
		Nb of cows	Nb of primiparous	Nb of multiparous	Parity avg	DIM	Nb of cows	Nb of primiparous	Nb of multiparous	Parity avg	DIM
Farm 1	Ctrl	39	10	29	2.3	16	28	8	20	2.3	47
	Exp	39	10	29	2.3	18	28	8	20	2.3	46
	Int. A	23	6	17	3.0	11	30	7	23	3.1	44
	Int. B	7	0	7	3.4	16	18	7	11	2.4	44
Farm 2	Ctrl	/					4	1	3	2.3	46
	Exp						6	0	6	3.0	54
	Int. A						6	0	6	3.2	50
	Int. B						6	1	5	2.8	46
Farm 3	Ctrl	9	1	8	2.9	20	/				
	Exp	9	1	8	2.9	19					
	Int. A	8	4	4	1.6	14					
	Int. B	2	0	2	5.0	21					

A. How are different dosage and time of giving of MecoVit® impacting the milk yield in early lactation in Poland (<60 days of lactation)?

In this part, the following data from milk recordings are studied: Milk yield, Fat percentage and Protein percentage. In addition to these data, the fat and protein production in kg/d and the Energy Corrected Milk (ECM) were calculated and used as data. The following equations were used:

Fat production (kg/d) = Milk yield (kg/d) * Fat (%) / 100

Protein production (kg/d) = Milk yield (kg/d) * Protein (%) / 100

ECM (kg/d) = Milk yield (kg/d) * ((0.383 * Fat (%) + 0.242 * Protein (%) + 0.7832) / 3.1138)

For each of the 3 farms, the mean of these data for each group are presented with the standard deviation. The standard deviation is the square root of the variance and is a measure of the dispersion of the values in the statistical sample. These descriptive statistics allow a comparison of the production trends of the different groups and thus compare the effect of the use of the MecoVit® product on milk production.

Next, the results of statistics studying the effect of parity and season are presented. To study these factors, 2-way anova tests are performed. These tests take into account the effect of the group (MecoVit® treatment or not) and the effect of the season but also the effect of the interaction of these 2 factors. The same is done for the study of the effect of parity, knowing that for this test only multiparous cows were kept since the production between a cow in first lactation and in second or more is already very different without taking into account the MecoVit® treatment. The interest was to see if there was an effect of parity only between the cows already multiparous. The results of these 2-way anova test are presented through the sum squares and the p-value ("sig"column). The sum of squares is the variation between the group means and the overall means and the p-value is the probability of obtaining a value at least as extreme as that observed under the null hypothesis.

Then a t-test was performed to see if the difference in production criteria was significantly different between the groups. The choice was made to focus on the control and experimental groups only as these are the two groups of real interest to compare as the experimental group contains cows that received the recommended dosage of MecoVit® (case 1 or 2) and the control group those that did not receive MecoVit® at all. In addition, the control and experimental groups on each farm are often the most comparable in terms of number of animals, parity and days in milk, as in the intermediate groups there were not always enough animals available (depending on calving dates). For these t-tests, a confidence level of 95% was chosen, which means that the difference between the two groups is considered significant when the p-value (column "sig") is less than 0.05.

On this part, a table for each farm will also present the average milk yield and ECM between the control and experimental groups divided according to whether the individuals were primiparous or multiparous. This table is presented only for farm 1 due to the lack of individuals on the other farms as explained above.

a. Farm 1

The results presented in table 14 show that MecoVit® supplementation tends to increase milk yield by slightly decreasing the percentage of fat and protein. However, fat and protein production calculated in kg per day seem to be increased by MecoVit® supplementation and the same is found when calculating the ECM. Furthermore, the intermediate groups had better results than the experimental group.

Table 14: Mean +/- standard deviation of milk yield, fat percentage and production, protein percentage and production and energy corrected milk according to treatment groups (farm 1)

	< 30 days in milk				< 60 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Milk yield (kg/d)	36.3 +/- 7.7	40.1 +/- 7.2	39.2 +/- 8.2	45.5 +/- 6.1	39.3 +/- 6.8	42.3 +/- 10.9	47.2 +/- 7.7	44.4 +/- 6.9
Fat (%)	4.42 +/- 1.0	4.36 +/- 0.8	4.81 +/- 1.2	4.26 +/- 0.8	3.38 +/- 1.0	3.81 +/- 0.6	3.62 +/- 0.5	3.71 +/- 0.7
Fat (kg/d)	1.58 +/- 0.5	1.74 +/- 0.4	1.87 +/- 0.7	1.93 +/- 0.4	1.32 +/- 0.4	1.61 +/- 0.5	1.70 +/- 0.3	1.65 +/- 0.4
Protein (%)	3.48 +/- 0.5	3.35 +/- 0.4	3.55 +/- 0.4	3.21 +/- 0.2	3.10 +/- 0.3	3.06 +/- 0.3	3.11 +/- 0.2	3.00 +/- 0.2
Protein (kg/d)	1.25 +/- 0.2	1.32 +/- 0.2	1.37 +/- 0.3	1.44 +/- 0.1	1.21 +/- 0.2	1.28 +/- 0.3	1.46 +/- 0.2	1.30 +/- 0.2
ECM (kg/d)	38.3 +/- 8.5	41.8 +/- 7.8	43.5 +/- 10.0	46.4 +/- 6.0	35.5 +/- 7.0	40.3 +/- 10.4	44.2 +/- 7.1	41.7 +/- 7.4

The results of the study of the effect of parity and season are presented in tables 15 and 16. In order to analyse the results of a 2-way anova test, first the effect of the interaction of the 2 factors (parity and group or season and group) should be analysed. Here, this interaction of 2 factors has mostly no effect on the value of the milk production and the percentage of fat and protein because the p-values are higher than the 0.05 threshold. The only exception is the effect of parity-group interaction on fat percentage in the first control (>30d) (p-value of 0.013). Having seen that the effect of interaction is almost systematically non-significant, it is then possible to look separately at the effects of the other single variables (parity, group, season). In the same way, p-values above 0.05 indicate a non-significant effect of these variables on the quantity of milk and the percentage of fat and protein.

Table 15 : Results of 2 way anova test of parity and group effects (farm 1)

		< 30 days in milk		< 60 days in milk	
		Sum squares	**Sig**	**Sum squares**	**Sig**
Milk yield (kg/d)	**Group**	261.332	**.157**	588.320	**.061**
	Parity	94.058	**.589**	85.647	**.887**
	Group*Parity	226.500	**.857**	210.734	**.968**
Fat (%)	**Group**	9.576	**.017**	3.684	**.059**
	Parity	14.768	**.002**	2.987	**.188**
	Group*Parity	20.239	**.013**	6.213	**.180**
Protein (%)	**Group**	.714	**.339**	.286	**.193**
	Parity	.864	**.256**	.286	**.312**
	Group*Parity	1.002	**.845**	.371	**.703**

Table 16: Results of 2 way anova test of season and group effects (farm 1)

		< 30 days in milk		< 60 days in milk	
		Sum squares	**Sig**	**Sum squares**	**Sig**
Milk yield (kg/d)	**Group**	213.986	**.162**	88.071	**.534**
	Season	12.742	**.640**	52.931	**.386**
	Group*Season	.000	**.**	.000	**.**
Fat (%)	**Group**	2.559	**.260**	.119	**.892**
	Season	.554	**.444**	.028	**.818**
	Group*Season	.000	**.**	.000	**.**
Protein (%)	**Group**	.729	**.157**	.301	**.068**
	Season	.190	**.324**	.006	**.736**
	Group*Season	.000	**.**	.000	**.**

The results of the t-test table 17 show that only the milk production in the first milk recording (<30d) and the fat production in the second milk recording (<60d) are significantly different between the control and the experimental group (p-value 0.028 and 0.019 respectively).

Table 17: Results of t-test between control and experimental groups concerning following parameters: yield, fat, protein and energy corrected milk (farm 1)

	< 30 days in milk			< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**	**Sig**	**Mean diff**	**Std Error Diff**
Milk yield (kg/d)	**.028**	-3.8000	1.6938	**.221**	-2.9893	2.4162
Fat (%)	**.770**	.06051	.20604	**.059**	-.41750	.21614
Fat (kg/d)	**.128**	-.15462	.10037	**.019**	-.28714	.11875
Protein (%)	**.224**	.12692	.10349	**.541**	.04214	.06847
Protein (kg/d)	**.123**	-.07897	.05063	**.329**	-.06964	.07076
ECM (kg/d)	**.064**	-3.47179	1.84381	**.064**	-3.47179	1.84381

In table 18 the results for primiparous and multiparous cows are presented separately, and it is noticeable that the increase in milk yield following MecoVit® supplementation is even more marked for primiparous cows.

Table 18: Mean +/- standard deviation of milk yield and energy corrected milk between control and experimental group and between primiparous and multiparous (farm 1)

	< 30 days in milk				< 60 days in milk			
Parity	**Primiparous**		**Multiparous**		**Primiparous**		**Multiparous**	
Group	**Ctrl**	**Exp**	**Ctrl**	**Exp**	**Ctrl**	**Exp**	**Ctrl**	**Exp**
Milk yield (kg/d)	29.8 +/- 1.7	35.3 +/- 7.1	38.5 +/- 7.7	41.7 +/- 6.6	33.5 +/- 5.6	37.6 +/- 4.5	41.6 +/- 5.8	44.1 +/- 12.1
ECM (kg/d)	30.8 +/- 1.8	35.4 +/- 6.8	40.9 +/- 8.3	44.0 +/- 6.9	32.6 +/- 8.5	35.4 +/- 3.9	36.7 +/- 6.2	42.3+/- 11.6

b. Farm 2

On this second farm, the trends are a bit different than on the first, as the experimental group showed an increase in milk yield but a decrease in ECM and fat production. As on the first farm, the intermediate groups seem to show better results.

Table 19: Mean +/- standard deviation of milk yield, fat percentage and production, protein percentage and production and energy corrected milk according to treatment groups (farm 2)

	< 60 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Milk yield (kg/d)	41.4 +/- 5.1	45.4 +/- 8.0	45.6 +/- 8.0	46.5 +/- 9.2
Fat (%)	3.39 +/- 0.4	2.89 +/- 0.9	3.49 +/- 0.5	3.2 +/- 0.5
Fat (kg/d)	1.39 +/- 0.1	1.27 +/- 0.3	1.59 +/- 0.4	1.45 +/- 0.2
Protein (%)	3.01 +/- 0.2	2.90 +/- 0.2	2.86 +/- 0.2	3.22 +/- 0.1
Protein (kg/d)	1.25 +/- 0.2	1.31 +/- 0.2	1.30 +/- 0.2	1.49 +/- 0.3
ECM (kg/d)	37.3 +/- 3.4	37.2 +/- 5.2	41.2 +/- 7.8	41.1 +/- 6.3

The results of the 2-way anova tests show a non-significant effect of parity (excluding primiparous cows), season and group on milk production and fat and protein percentages except for a significant effect of group and parity interaction on milk production in the first milk recording.

Table 20: Results of 2 way anova test of parity and group effects (farm 2)

		< 60 days in milk	
		Sum squares	**Sig**
Milk yield (kg/d)	**Group**	173.370	**.184**
	Parity	203.288	**.220**
	Group*Parity	523.105	**.015**
Fat (%)	**Group**	.481	**.805**
	Parity	.387	**.933**
	Group*Parity	2.314	**.261**
Protein (%)	**Group**	.408	**.077**
	Parity	.160	**.485**
	Group*Parity	.098	**.541**

Table 21 : Results of 2 way anova test of season and group effects (farm 2)

		< 60 days in milk	
		Sum squares	**Sig**
Milk yield (kg/d)	**Group**	133.997	**.559**
	Season	66.667	**.317**
	Group*Season	.000	**.**
Fat (%)	**Group**	1.480	**.352**
	Season	.360	**.369**
	Group*Season	.000	**.**
Protein (%)	**Group**	.452	**.034**
	Season	6.667E-5	**.968**
	Group*Season	.000	**.**

The t-test results presented in table 22 show that there is no significant effect (p-value above 0.05) of MecoVit® supplementation on the milk production criteria studied despite the trends presented in table 19.

Table 22 : Results of t-test between control and experimental groups concerning following parameters: yield, fat, protein and energy corrected milk (farm 2)

	< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**
Milk yield (kg/d)	**.404**	-4.0000	4.54102
Fat (%)	**.346**	.50333	.5097
Fat (kg/d)	**.480**	.11917	.16070
Protein (%)	**.416**	.12250	.14286
Protein (kg/d)	**.690**	-.05583	.13481
ECM (kg/d)	**.985**	.05833	2.96925

c. Farm 3

On this third farm, the trends following MecoVit® supplementation are as follows: an increase in milk yield, a decrease in fat percentage but an increase in kg/d, an increase in protein percentage and production and an increase in ECM. The results are better in the intermediate B group.

Table 23 : Mean +/- standard deviation of milk yield, fat percentage and production, protein percentage and production and energy corrected milk according to treatment groups (farm 3)

	< 30 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Milk yield (kg/d)	39.5 +/- 12.0	46.6 +/- 12.6	32.8 +/- 10.5	57.7 +/- 8.8
Fat (%)	4.11 +/- 0.7	3.82 +/- 0.7	3.73 +/- 0.5	4.08 +/- 0.8
Fat (kg/d)	1.60 +/- 0.4	1.75 +/- 0.5	1.19 +/- 0.3	2.39 +/- 0.8
Protein (%)	3.20 +/- 0.3	3.32 +/- 0.4	3.62 +/- 0.5	2.94 +/- 0.1
Protein (kg/d)	1.26 +/- 0.4	1.53 +/- 0.4	1.17 +/- 0.3	1.69 +/- 0.2
ECM (kg/d)	39.4 +/- 10.7	45.1 +/- 11.2	31.0 +/- 8.6	57.1 +/- 14.2

According to the results presented in tables 24 and 25, there is no significant effect of season, group and parity on milk production and percentage of fat and protein.

Table 24 : Results of 2 way anova test of parity and group effects (farm 1)

		< 30 days in milk	
		Sum squares	**Sig**
Milk yield (kg/d)	**Group**	39.362	**.787**
	Parity	649.487	**.098**
	Group*Parity	394.837	**.354**
Fat (%)	**Group**	.534	**.670**
	Parity	1.441	**.545**
	Group*Parity	.419	**.953**
Protein (%)	**Group**	.534	**.670**
	Parity	1.441	**.545**
	Group*Parity	.419	**.953**

Table 25 : Results of 2 way anova test of season and group effects (farm 3)

		< 30 days in milk	
		Sum squares	**Sig**
Milk yield (kg/d)	**Group**	1440.994	**.034**
	Season	55.125	**.536**
	Group*Season	.000	**.**
Fat (%)	**Group**	.884	**.573**
	Season	.342	**.384**
	Group*Season	.000	**.**
Protein (%)	**Group**	.884	**.573**
	Season	.342	**.384**
	Group*Season	.000	**.**

The results of the t-test between cows supplemented with MecoVit® (experimental) and not supplemented with MecoVit® (control) reveal that there are no significant differences in terms of milk production and milk fat and protein.

Table 26 : Results of t-test between control and experimental groups concerning following parameters: yield, fat, protein and energy corrected milk (farm 3)

	< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**
Milk yield (kg/d)	**.239**	-7.08889	5.79040
Fat (%)	**.391**	0.28778	0.32674
Fat (kg/d)	**.497**	-0.14667	0.21092
Protein (%)	**.464**	-0.11889	0.15845
Protein (kg/d)	**.176**	-0.26111	0.18439
ECM (kg/d)	**.288**	-5.65556	5.14998

B. How are different dosage and time of giving of MecoVit® impacting the milk quality parameters in early lactation in Poland (<60 days of lactation)?

In this section, the milk quality criteria studied are casein and lactose percentage, somatic cell count (SCC) and urea content. A summary table of averages and standard deviations for each treatment group is presented for each farm to observe the evolution trends of these parameters due to the supplementation with MecoVit®. Then the choice was made to also observe the median for the somatic cell parameters since the mean may not be very representative in case some cows present extremely high values. Finally for each farm a t-test will be performed to determine if the differences in values for these different criteria are significantly different between the experimental group and the control group.

a. Farm 1

On the first farm, the trends following MecoVit® supplementation were as follows: decrease in casein percentage, stability in lactose percentage, increase in SCC and decrease in urea on the first test but increase on the second test.

Table 27 : Mean +/- standard deviation of casein, lactose, SCC and urea according to treatment groups (farm 1)

	< 30 days in milk				**< 60 days in milk**			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Casein (%)	2.68 +/- 0.4	2.61 +/- 0.3	2.75 +/- 0.3	2.51 +/- 0.2	2.47 +/- 0.5	2.37 +/- 0.2	2.45 +/- 0.2	2.32 +/- 0.2
Latose (%)	4.81 +/- 0.2	4.81 +/- 0.2	4.81 +/- 0.2	4.81 +/- 0.2	4.83 +/- 0.2	4.85 +/- 0.1	4.92 +/- 0.2	4.84 +/- 0.1
SCC	275 +/- 677	363 +/- 744	274 +/- 479	856 +/- 1713	223 +/- 801	195 +/- 349	499 +/- 1808	333 +/- 611
Urea	237 +/- 70	220 +/- 57	179 +/- 66	187 +/- 24	249 +/- 76	289 +/- 73	217 +/- 45	242 +/- 51

If the median SCC is studied, then there is an increase in SSC following MecoVit® supplementation but it is not a major increase (Table 28).

Table 28: Median of SCC according to treatment groups (farm 1)

	< 30 days in milk				< 60 days in milk			
Group	Ctrl	Exp	Int. A	Int. B	Ctrl	Exp	Int. A	Int. B
SCC	62	80	97	33	35	36	61	55

The t-tests show non-significant differences in these milk quality parameters between the control and experimental groups (Table 29).

Table 29: Results of t-test between control and experimental groups concerning following parameters: casein, lactose, SCC and urea (farm 1)

	< 30 days in milk			**< 60 days in milk**		
	Sig	**Mean diff**	**Std Error Diff**	**Sig**	**Mean diff**	**Std Error Diff**
Casein (%)	**,338**	,08026	,08326	**,241**	,11179	,09425
Latose (%)	**,944**	-,00256	,03664	**,646**	-,01821	,03942
SCC	**,585**	-88,30769	161,10482	**,871**	27,03571	165,22568
Urea	**,245**	16,97436	14,48165	**,049**	-40,28571	19,98455

b. Farm 2

On the second farm, MecoVit® supplementation seems to result in the same trends for casein and lactose: a decrease in casein percentage, stability of lactose percentage (or a slight increase). On the other hand, the SCC decreased as well as the amount of urea (Table 30).

Table 30 : Mean +/- standard deviation of casein, lactose, SCC and urea according to treatment groups (farm 2)

	< 60 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Casein (%)	2.34 +/- 0.2	2.26 +/- 0.2	2.21 +/- 0.2	2.50 +/- 0.1
Latose (%)	4.86 +/- 0.1	4.94 +/- 0.1	4.87 +/- 0.2	4.88 +/- 0.1
SCC	373 +/- 686	205 +/- 414	24 +/- 20	39 +/- 56
Urea	247 +/- 76	187 +/- 43	203 +/- 86	204 +/- 39

The median number of somatic cells also decreased (Table 31).

Table 31 : Median of SCC according to treatment groups (farm 2)

	< 60 days in milk			
Group	Ctrl	Exp	Int. A	Int. B
SCC	38	30	18	14

As on the first farm studied, the t-tests show non-significant differences in these milk quality parameters between the control and experimental groups (Table 32).

Table 32 : Results of t-test between control and experimental groups concerning following parameters: casein, lactose, SCC and urea (farm 2)

	< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**
Casein (%)	**,552**	,07333	,11801
Latose (%)	**,375**	-,07667	,08169
SCC	**,639**	167,50000	343,56836
Urea	**,146**	60,00000	37,22853

c. Farm 3

In this farm, the percentage of casein and lactose increased with MecoVit® supplementation (except for the intermediate B group). Also, the SCC decreased, and the urea level decreased in the MecoVit® supplemented groups.

Table 33 : Mean +/- standard deviation of casein, lactose, SCC and urea according to treatment groups (farm 3)

	< 30 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Casein (%)	2.48 +/- 0.2	2.52 +/- 0.3	2.80 +/- 0.4	2.24 +/- 0.1
Latose (%)	4.77 +/- 0.2	4.80 +/- 0.2	4.79 +/- 0.2	4.73 +/- 0.1
SCC	924 +/- 1743	135 +/- 168	57 +/- 44	462 +/- 615
Urea	289 +/- 82	254 +/- 58	213 +/- 88	273 +/- 115

With the exception of the intermediate B group, SSC decreased in the MecoVit® supplemented groups compared to the control group (Table 34).

Table 34 : Median of SCC according to treatment groups (farm 1)

	< 30 days in milk			
Group	Ctrl	Exp	Int. A	Int. B
SCC	202	42	48	462

As for the two previous farms, the t-tests show non-significant differences in these milk quality parameters between the control and experimental groups (Table 35).

Table 35 : Results of t-test between control and experimental groups concerning following parameters: casein, lactose, SCC and urea (farm 3)

	< 30 days in milk		
	Sig	**Mean diff**	**Std Error Diff**
Casein (%)	**,755**	-0,4000	,12618
Latose (%)	**,718**	-,03556	,09691
SCC	**,195**	789,55556	583,62468
Urea	**,306**	35,44444	33,53706

C. How are different dosage and time of giving of MecoVit® impacting the cows' health and particularly the risk of ketosis in early lactation in Poland (<60 days of lactation)?

In this section, the fat/protein ratio is used to study the risk of ketosis because it is considered that above the threshold of 1.4 for this ratio, there is a risk of ketosis in the dairy cow. For each farm the average of this ratio in each group and the number of cows above the threshold of 1.4 are presented in a first table. Next, the results of the t-test between the control and experimental group define whether the difference in this ratio is significant between these two groups.

a. Farm 1

The averages show an increase in the fat/protein ratio in the MecoVit® supplemented groups but the number of cows above 1.4 decreased in these groups (Table 36).

Table 36: Mean +/- standard deviation of fat/protein ratio according to treatment groups and number of cows above the threshold of 1.4 (farm 1)

	< 30 days in milk				**< 60 days in milk**			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Ratio fat/protein	1.25 +/- 0.2	1.31 +/- 0.2	1.36 +/- 0.3	1.33 +/- 0.2	1.11 +/- 0.3	1.25 +/- 0.2	1.17 +/- 0.2	1.27 +/- 0.2
Cows with a ratio above 1.4	2	1	0	0	1	0	0	0

The p-value greater than 0.05 shows that the difference in this ratio between the control group and the experimental group is not significant (Table 37).

Table 37: Results of t-test between control and experimental groups concerning fat/protein ration (farm 1)

	< 30 days in milk			< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**	**Sig**	**Mean diff**	**Std Error Diff**
Ratio fat/protein	**,314**	-,05494	,05414	**,049**	-,13538	,06730

b. Farm 2

On this second farm the ratio decreased this time in the supplemented groups, as did the number of cows with a ratio above 1.4 (Table 38). However, the difference in ratio between the experimental and control group is not significant according to the results in table 39.

Table 38 : Mean +/- standard deviation of fat/protein ratio according to treatment groups and number of cows above the threshold of 1.4 (farm 1)

	< 60 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Ratio fat/protein	1.13 +/- 0.2	0.99 +/- 0.3	1.23 +/- 0.2	0.98 +/- 0.1
Cows with a ratio above 1.4	3	1	0	0

Table 39 : Results of t-test between control and experimental groups concerning fat/protein ration (farm 2)

	< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**
Ratio fat/protein	**,357**	,1433	,14665

c. Farm 3

The results for this third farm follow the same trends as for farm 2 (Table 40 and 41).

Table 40 : Mean +/- standard deviation of fat/protein ratio according to treatment groups and number of cows above the threshold of 1.4 (farm 3)

	< 30 days in milk			
Group	**Ctrl**	**Exp**	**Int. A**	**Int. B**
Ratio fat/protein	1.29 +/- 0.3	1.17 +/- 0.3	1.04 +/- 0.1	1.40 +/- 0.3
Cows with a ratio above 1.4	3	1	0	1

Table 41 : Results of t-test between control and experimental groups concerning fat/protein ration (farm 1)

	< 60 days in milk		
	Sig	**Mean diff**	**Std Error Diff**
Ratio fat/protein	**,322**	,12889	,12622

The results show that MecoVit® supplementation leads to similar trends on these 3 farms. Indeed, the milk yield systematically increases following the supplementation. The percentage of fat and protein tended to decrease but when the production of fat and protein was calculated in kg per day, the trends were more upward for the supplemented cows. As for the other milk parameters, the results on the different farms and at the first or second check are more disparate. Indeed, the percentage of casein in the supplemented cows tends to decrease in the first two farms while it increases in the third. For lactose there is little difference between the control group and the other groups but the trends are sometimes a slight increase and sometimes a slight decrease. Concerning SCC, it is lower in the supplemented groups in farms 2 and 3 but higher in the supplemented groups in the recording before day 30 of lactation in the first farm. The same is true for urea, which is lower in farms 2 and 3 but higher in the supplemented groups on the first farm at the second recording this time. Finally, concerning the fat/protein ratio, the difference between the control and the experimental group is never significant but the trend is a lower ratio in the supplemented cows in farms 2 and 3 while this ratio is higher in the supplemented cows in farm 1. However, the number of cows above 1.4 is always lower in the MecoVit® supplemented groups on all farms. On the three farms the results are most of the time not statistically significant on all these milk production criteria studied (except for the milk yield on farm 1 where the difference between the control and experimental groups was significant), but on the farm 2 and 3 it can be due to a low number of cows on the farm so a low number if individuals in each group compared, which leads to a lower statistical power.

Chapter 4: Discussion

A. Discussion of the results compared to scientific literature

a. How are different dosage and time of giving of MecoVit® impacting the milk yield in early lactation in Poland (<60 days of lactation)?

MecoVit® supplementation resulted in increased milk production during early lactation (2 first 2 recordings) in all 3 farms studied. This is in line with the results of the scientific literature that have shown that supplementation with methionine (Cetin *et al.*, 2018) (Vailati-Riboni *et al.*, 2016), choline (Erdman & Sharma, 1991) (Santos & Lima, n.d.) and betaine (Monteiro et al., 2016) leads to increased milk production. Since MecoVit® combines the supplementation of these 3 elements, the increase in milk production seems consistent. However, the difference in production between the control group and the experimental group in the first milk recording was 3.8kg (farm 1) and 7.1kg (farm 3) and in the second milk recording 3kg (farm 1) and 4kg (farm 2), so these differences are probably not only related to MecoVit® supplementation but also to other factors (ration, environment, individuals in each group) (see paragraph on the discussion of the materials and methods).

The results on farm 1 clearly show that MecoVit® supplementation leads to a higher increase of milk yield in primiparous cows. In the first control, primiparous cows in the experimental group produced 5.5 kg more milk compared to 3.2 kg more milk for multiparous cows in the experimental group (compared to the respective control group). Similarly, in the second recording (between 30 and 60 days of lactation), there was a difference of 4.1 kg between the production of primiparous cows in the experimental group and those in the control group, compared to 2.5 kg between multiparous cows in the experimental and control groups. A scientific paper has also shown this different response to choline and methionine supplementation depending on parity (Potts *et al.*, 2020). In this study, choline supplementation was combined or not with methionine supplementation. The results show that choline supplementation increases milk production in primiparous cows by 3.5 kg whether or not it is combined with methionine supplementation. However, multiparous cows showed an increase in milk production following choline supplementation but only in the absence of methionine. Here choline and methionine were distributed in the single MecoVit® product so the experiment was different but it was proven that primiparous and multiparous cows had a different response to combined choline and methionine supplementation in terms of milk production.

Furthermore, in the results obtained, the milk production of the intermediate groups (i.e. those that did not receive MecoVit® supplementation during the whole trial period) is sometimes better, as the results from farm 1 clearly show. Indeed, the intermediate B group had better results in terms of milk production compared to the experimental group which had received MecoVit® during the whole recommended period (close up and fresh period in this case). However, these results can be explained by a higher parity in the intermediate B group compared to the experimental group. Indeed the parity of the experimental group is 2.3 compared to 3.4 and 2.4 for the intermediate B group of the 1st and 2nd recordings respectively. Also the number of cows in the intermediate B group for this farm is lower than in the control group (7 cows for the first recording and 18 for the second recording compared to 39 and 28 for the experimental groups of the first and second recordings). However, a lower number of individuals in a group gives more reliable results statistically since the more individuals there are in our statistical sample, the more representative it is of the population.

Concerning the results of the evolution of milk fat and protein, the differences between the control and experimental groups are never significant. However, the trend is a decrease in percentage but an increase in terms of production in kg per day of this fat and protein. These results are quite controversial in the scientific literature but other papers also find a decrease in the percentage of fat and protein following combined methionine and choline supplementation (Cetin *et al.*, 2018). This difference shown in table 42 is not significant as was found in the results shown in the previous section (Cetin *et al.*, 2018). For this paper the fat and protein production are not presented but if calculated we obtain the results presented in table 43. The results of the Cetin *et al.* (2018) study are in line with those presented in the results section, i.e. a decrease in fat and protein percentage but an increase in fat and protein production in kg/d due to an increase in milk production.

Table 42: Effect of supplementing Holstein cows during peripartal period with methionine, rumen protected choline or both on DMI, milk yield and milk composition (Cetin et al., 2018)

Parameters	**CON X±Sx**	**MET X±Sx**	**CHOL X±Sx**	**MIX X±Sx**
Close-up DMI (kg/d)	14.70±2.66	14.79±3.24	13.99±2.82	13.92±3.55
Lactation DMI (kg/d)	21.17±4.4^{b}	21.89±4.12^{a}	19.90±4.27^{c}	22.01±4.13^{a}
Milk yield (kg/d)	40.85±5.5^{c}	42.88±4.72^{a}	40.05±5.93^{c}	42.00±5.09^{b}
Milk fat (%)	3.80±1.07^{b}	4.02±1.20^{a}	3.49±1.13^{c}	3.73±1.05^{b}
Milk protein (%)	3.06±0.37^{b}	3.17±0.35^{a}	2.93±0.43^{c}	3.04±0.32^{b}
Milk lactose (%)	4.61±0.16^{b}	4.74±0.20^{a}	4.63±0.22^{b}	4.63±0.17^{b}
Solids non fat(%)	8.51±0.49^{b}	8.76±0.48^{a}	8.38±0.49^{c}	8.51±0.40^{b}
Total solids (%)	12.42±1.3^{b}	12.93±1.46^{a}	11.93±1,42^{c}	12.35±1.30^{b}
Milk urea nitrogen (mg/dL)	14.45±3.73	14.30±3.54	14.27±2.94	14.26±3.29
Different superscripts indicate stastical differences ^{a,c} P<0.028				

Table 43: Calculation of fat and protein production from fat percentage and milk yield obtained in Cetin et al. study (2018)

	CON	**MIX**
Fat production (kg/d)	1.55	1.57
Protein production (kg/d)	1.25	1.28

However, these results must be put into perspective since farmers are not very sensitive to the notion of fat and protein production in kg per day, as they pay attention to the percentage since the dairy applies penalties according to the value of these percentages. Indeed, following a discussion with the farmer of farm 1, he explained that the dairy to which he sells does not apply a penalty on the protein percentage but applies a penalty in case the fat percentage is lower than 3.7%. However, there is no benefit in producing milk with more than 3.7% fat because there is no additional promotion. The objective of this farmer is therefore to maintain the 3.7% fat level while increasing milk production. This is achieved by using MecoVit®, which allows a better milk yield in the experimental group compared to the control group (+3.8 kg/d in the first control and +3kg/d in the second control). In addition to this, the use of MecoVit® does not lower the fat percentage below 3.7 and even allows this threshold to be reached since in the results of the second milk recording for farm 1, the fat percentage was 3.38% for the control group and 3.81% for the experimental group.

b. How are different dosage and time of giving of MecoVit® impacting the milk quality parameters in early lactation in Poland (<60 days of lactation)?

The results for casein percentage, lactose percentage, SCC and urea level show that MecoVit® supplementation never significantly affects these parameters as the p-values of the t-tests were systematically higher than 0.05. The study by Ye *et al.* (2010) showed similar results as different supplementations were tested: lysine, methionine and choline, and the percentage of lactose and SSC were never significantly different between treatments. Another study showed that choline supplementation tended to decrease the percentage of lactose but that this decrease was not significant (Pirestani & Aghakhani, 2018). In fact, in figure 12 the percentage of lactose in the control group and the choline supplementation group (Ch) show overlapping error bars and the same letter "a" which prove that the difference is not significant.

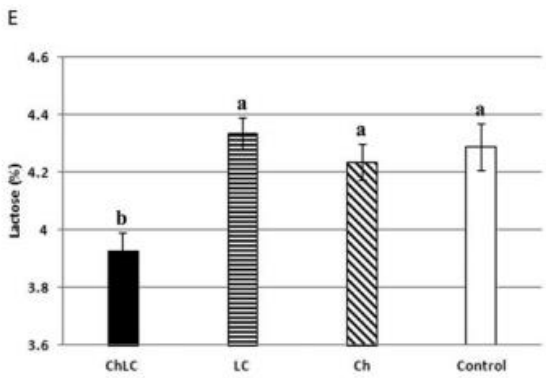


Figure 12: Lactose percentage in groups supplemented in choline and L-carnitine (Pirestani & Aghakhani, 2018)

Concerning the SCC, the article from Pirestani *et al.* (2011) highlights that protected choline supplementation of 60g/d allows lower SCC during the 3 first weeks after calving according to the results table 44.

Table 44: Comparison of average SCC in milk of different treatments in the five sampling times (Pirestani et al., 2011)

Treatment/Sample	Calving	*weeks after parturition*				Total average
		1	2	3	4	
Control	2209[a]	642/7[a]	788/4[a]	449/7[b]	144/6[a]	846/88
Choline	320[a]	134/6[a]	523/6[a]	408/2[ab]	348/1[a]	346/9
L-carnitine	239[a]	833/9[a]	356/9[a]	751/9[a]	241/9[a]	484/72
Choline +L-carnitine	436[a]	602/6[a]	909/6[a]	348/9[ab]	132/2[a]	485/86
Standard error	980/56	275/5	419/26	200/24	84/21	

* In each column mean which contained at least one common letter are not significantly different at the 5% level.

A decrease in SCC was demonstrated on farms 2 and 3 but on farm 1 there was an increase in SCC in the MecoVit® supplemented groups at the first check. However, following a discussion with the farmer, he informed us that he had encountered a bedding problem during this period of the trial so this increase in somatic cells in the experimental group may be due to other factors such as environmental factors. It should be remembered that the control group and the experimental group did not come from the same period of the year as the trial was an off/on trial and not a trial with a parallel control and experimental group. This test mode will be discussed in the next section.

Regarding urea levels, results following protected methionine supplementation showed a decrease in urea levels (180.8 mg/L compared with CTR1 193.0 mg/L and CRT2 189.9 mg/L) (Sainz de la Maza *et al.,* 2019). Similar results were found in farms 2 and 3 with a tendency to decrease urea levels in the MecoVit® supplemented groups although the differences were not significant. On farm 1 the urea level was also lower in the supplemented groups than in the control group on the first milk recording but on the second milk recording the experimental group had a higher urea level than the control group. According to the farmer, this can be explained by a slight change in the ration at the time the experimental group was studied, which is again due to the off/on mode of operation of this trial.

Vetagro has already conducted trials on the supplementation of protected methionine with the product Timet and the results are presented in table 45 (Vetagro, 2019). These results show the same trends as those found in the MecoVit® supplementation trial (which combines methionine with choline, betaine and vitamin B). Indeed, the results show an increase in milk yield and ECM, a very slight increase in protein percentage (for supplementation above 20g) and a decrease in fat percentage but an increase in fat and protein production in kg per day premised on the increase in milk yield. As with MecoVit® supplementation, Timet supplementation does not result in a significant difference in lactose percentage or SCC.

Table 45: Performance of 48 Holstein dairy cows (with an average of 100 days in milk) consuming Timet during 28 days (Vetagro, 2019)

	Dietary Treatment[1]				SEM[2]	*P*-value[3]			
	CTR	T20	T35	T50		Trt	Lin	Quad	Cubic
Dry Matter Intake, kg/day	23.59	23.39	23.02	23.33	0.46	0.64	0.39	0.49	0.48
Milk Yield, kg/day	38.05	39.31	39.16	39.73	0.87	0.11	0.03	0.53	0.41
3.5% FCM[4]	37.77[b]	40.88[a]	40.15[a]	40.90[a]	1.08	0.01	0.007	0.13	0.17
ECM[5]	38.35[b]	41.45[a]	40.90[a]	41.67[a]	1.05	0.009	0.004	0.14	0.21
Protein, %	3.17	3.16	3.22	3.21	0.04	0.18	0.11	0.66	0.15
Protein, kg	1.16[b]	1.24[a]	1.25[a]	1.27[a]	0.03	0.003	0.0006	0.23	0.36
Fat, %	3.72	3.69	3.70	3.66	0.88	0.83	0.42	0.87	0.67
Fat, kg	1.35[b]	1.45[a]	1.43[a]	1.45[a]	0.05	0.03	0.02	0.14	0.25
Lactose, %	4.66	4.63	4.67	4.62	0.03	0.35	0.37	0.69	0.13
Lactose, kg	1.71[b]	1.85[a]	1.82[a]	1.86[a]	0.05	0.02	0.009	0.17	0.24
MUN, mg/dL	13.58	13.55	13.71	13.32	0.23	0.58	0.41	0.38	0.49
Somatic Cell Count	281.42	394.01	222.94	388.52	149.48	0.58	0.73	0.87	0.18
Body Weight, kg	672.82	671.48	670.03	671.54	8.51	0.82	0.54	0.53	0.69
Body Condition Score[6]	3.34	3.33	3.32	3.32	0.03	0.83	0.40	0.69	0.99

[1]**CTR** = Control; **T20** = CTR + Timet® 20 g; **T35** = CTR + Timet® 35 g; **T50** = CTR + Timet® 50 g
[2]Standard error of treatment mean is shown.
[3]Main effect of treatment.
[4]3.5% Fat corrected milk = [milk fat (kg) × 16.216] + [milk yield (kg) × 0.4324].
[5]ECM = [0.327 × milk yield (kg)] + [12.95 × milk fat (kg)] + [7.65 × milk protein (kg)].
[6]Body condition scores 1-5 scale (Wildman et al., 1982).
[a-d]Values in the same row with different superscript differ significantly.

c. How are different dosage and time of giving of MecoVit® impacting the cows' health and particularly the risk of ketosis in early lactation in Poland (<60 days of lactation)?

For this part the only data available to study this ketosis risk was the fat and protein ratio because not any data about the cows' health were available on these commercial farms. Looking at the results in the literature, methionine, betaine and choline supplementation allow a lower ketosis incidence (Table 44) (Davidson *et al.*, 2008). In this study from Davidson *et al.* (2008), the ketosis incidence is the number of cows with a BHBA concentration in plasma higher than 1.4 micromol/L.

Table 46: Plasma and serum metabolites as affected by dietary treatment for multiparous cows (Davidson et al., 2008)

	Dietary treatments[1]					
Item	Control	RP-MET	RP-BET	RP-CHOL	SEM	$P \leq$
SUN,[2] mg/d	14.9	14.6	16.1	15.3	0.8	0.62
Plasma NEFA, mEq/L	0.574	0.554	0.561	0.496	0.052	0.72
Serum total cholesterol,[3] mg/dL	191.2^{ab}	177.9^{b}	209.4^{a}	209.6^{a}	9.3	0.05
HDL, mg/dL	103.7	93.2	100.8	102.0	3.8	0.22
LDL,[4] mg/dL	84.6^{b}	82.5^{b}	109.5^{a}	105.5^{ab}	8.5	0.05
VLDL,[5] mg/dL	2.98	2.81	2.64	2.86	0.15	0.45
Serum triglycerides, mg/dL	14.9	14.1	13.2	14.3	0.7	0.45
BHBA, µmol/L	716	502	571	552	65	0.13
Ketosis incidence[6]	4	2	3	1	NA[7]	NA

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.

[2]SUN = serum urea N.

[3]HDL = high-density lipoproteins; LDL = low-density lipoproteins; VLDL = very low density lipoproteins.

[4]LDL (mg/dL) = cholesterol (mg/dL) − [HDL (mg/dL) + VLDL (mg/dL)] (Friedewald et al., 1972).

[5]VLDL (mg/dL) = triglycerides (mg/dL) ÷ 5 (Friedewald et al., 1972).

[6]Ketosis incidence is calculated as the number of cows from each treatment where BHBA > 1,400 µmol/L for any of the 4 sampling times (Duffield et al., 1997). Ketosis incidence data were not analyzed statistically, because the study was not designed to detect differences in the incidence of a disorder.

[7]NA = not applicable.

In this trial of MecoVit®, this BHBA concentration was not known so the fat/protein ratio was used as an indicator of ketosis incidence. Looking at the number of cows above 1.4 for this ratio, results show that MecoVit® supplementation allows to decrease this number of cows above this threshold.

The study of the impact of MecoVit® supplementation on cow health is very limited in our study due to the limited data available. However, MecoVit® is a product that should limit metabolic diseases after calving, so it would be very relevant to do a trial with more data available to study this health aspect more precisely. Also, better health after calving has an impact on production (which was studied in this trial) but also on reproduction performances. Thus, it would be very interesting to conduct a trial on farms that have reproductive performance data to study the effectiveness of MecoVit®.

B. Discussion of the materials and methods

Several aspects of the materials and methods can be discussed as they may jeopardise the reliability of the results obtained. First of all, there are fundamental differences in practices between the 3 farms studied. Indeed, the system can be a tethered cow system as well as a parlour system and different barns for each group of cows depending on the stage of lactation. Also on these three farms, the cows' rations are different as shown in table 12. The ration is the main factor affecting milk production, both in quantity and quality. For example, fat content is influenced by the amount of cellulose and sugar in the ration and protein content is influenced by the amount of crude protein available in the ration.

Concerning the experimental design, it would have been preferable to have a control group and an experimental group at the same time. In the method used, cows receiving MecoVit® were compared to cows that did not receive MecoVit® and therefore calved months before. This was because it was too complicated for the farmers to set up a MecoVit® and a non-MecoVit® group as it required extra work during ration distribution. Although the cows in the experimental group were chosen to have the same parity and lactation days as the cows in the control group, this does not prevent differences in production which may be due to factors other than the MecoVit® supplementation. Indeed, there may already be an effect of simply having different cows: there may be better producers without MecoVit® being the reason. There may also be a seasonal effect as the results of the cows in the control and experimental groups differ by several months (even if statistics seem to show that there is not). Also, incidents may occur on the farm at the time the experimental group is studied that were not present at the time the control group was studied (the litter problem on farm 1 is an example).

Regarding the available data, it would have been preferable to have the milk recording data at least every week. Here only the first two months of lactation are studied which means that only two controls per cow are studied which is very little to see an evolution.

Also, only these data from the milk recording were available, i.e. only parameters concerning milk production. However, it would have been interesting to have data on the health of the cows and on the reproductive performance. This would have required a longer trial period to see the effects of using MecoVit® and would also have required that this data was available on each farm which was not the case. Other data such as blood test results could have been of interest as this would have allowed analysis of the amount of NEFA, glucose and BHBA which are indicators of liver function and ketosis risk. BHBA is the major cetone body produced during ketosis so analysis of its concentration can determine whether the cow is in a ketosis situation or not. Portable devices can be easily used on the farm to measure BHBA in the blood. These devices use strips containing beta-hydroxybutyrate dehydrogenase. Also, it is possible to measure BHBA in milk or acetone in urine with very easy to use strips that are coloured according to the result. These kinds of practices could have been used in the protocol, they would have required additional work from the farmer but not too much. And if the farmer refused to take the time to do these measurements, it could have been done during the farm visits, which in this case should have been more regular.

Chapter 5: Conclusions and recommendations

The aim of this trial carried out on 18 Polish commercial farms was to determine how MecoVit® supplementation influences the start of lactation in terms of production and health of dairy cows. For this purpose, the results of the first two milk recordings after calving were analysed for cows not supplemented with MecoVit® (control group), supplemented with the recommended dosage during the whole recommended period (experimental group) or supplemented with MecoVit® during a partial part of the recommended period (intermediate group). In this report only the 3 farms that completed the trial period were studied, the others will be studied once their trial period is over. On these 3 farms, 2 different dosages were tested: 50g/cow/day during the close up and fresh period or 30g/cow/day during the entire dry period and lactation.

The results showed the influence of MecoVit® supplementation on milk quantity parameters, milk quality parameter and cow's health during early lactation, which are the 3 sub questions. First, concerning the quantity parameters following MecoVit® supplementation, the trend towards higher milk production is very clear. The results systematically show a better milk yield in the MecoVit® supplemented groups on all three farms. Concerning the fat and protein percentages, they tend to be slightly lower in the MecoVit® supplemented cows but when the fat and protein percentages produced per day are calculated, they are higher in the MecoVit® supplemented cows. Then, regarding other milk quality parameters in the MecoVit® supplemented groups, casein percentage tended to decrease slightly, lactose percentage increased slightly or remained the same, somatic cell count generally decreased and urea level also decreased. Finally, the fat to protein ratio was used as an indicator of cow health. Above the threshold of 1.4, cows were considered to be at risk of ketosis. The number of cows above this threshold was lower in the MecoVit® supplemented groups. The results obtained are mostly statistically not significant, but these trends are clearly apparent.

Regarding the different dosages and duration of MecoVit® supplementation tested, it is a bit difficult to come to certain conclusions on only 3 farms studied. Here 2 cases were studied as farm 1 and 3 met case 2, i.e. a supplementation of 50g/d/cow during the close up and fresh period and farm 2 met case 2 with a supplementation of 30g/d/cow during the whole dry period and lactating period. More reliable conclusions can be drawn once all farms have been studied, however the trends appear to be almost similar on all three farms and where there are differences, these appear to be explained by differences in feeding practices and not due to different dosage and time of supplementation with MecoVit®. If this is confirmed in the results of the other farms, it means that the dosage of 30g/d/cow applicable even in small farms without close up and fresh groups could give equally good results. Therefore, this trial proves to farmers the efficiency of MecoVit® used on different farms, with different dosage and with different time of giving. The target group will then choose the adapted dosage and time for their own farming system. The next steps in this trial are the analysis of all the results from the 18 farms in order to be able to create commercial documents for customers such as flyers or brochures. Also, the presentation of these results via a conference and a newspaper article will be part of the future steps. Also, other relevant tests could be carried out on this MecoVit® product to obtain even more precise results. For example, a trial with weekly milk recording data and with the presence of a control and experimental group in parallel (present simultaneously), could allow for more detailed results. Also, a trial over a longer period of time and on farms where data on the health and reproductive performance of the cows is available could allow to study the effect of MecoVit® supplementation on the post-calving health and reproductive capacity of dairy cows.

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