Effect of *Yucca Schidigera* inclusion in milk replacer for veal calves on health status, antimicrobial use and growth performance

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SUMMARY

Antimicrobial resistance is a global health problem. White veal calves face many challenges during the first period of life which, in combination with their immature physiological systems, may explain their high susceptibility to infections and increased use of antibiotics, mainly in the form of mass preventive treatments. The use of antibiotic alternatives, such as natural extracts, to improve calf immune function is gaining interest in rearing white veal calves.

This study evaluates the effect *Yucca Schidigera* inclusion in veal calf's milk replacer on immune functionality and growth performances.

The trial involved 1015 male Friesian calves divided in two groups: control (CON) and treatment (TREAT), differing for the inclusion of *Yucca Schidigera* extracts.

The zootechnical performances were evaluated: mortality, morbidity and slaughtering performances such as carcass weight, incidence of underweight carcasses, average daily gain and meat colour. At slaughterhouse, pulmonary score evaluation was performed to understand the severity and incidence of respiratory diseases. In terms of immune functionality and health status, the number, type and days of treatments were analysed. Also, on 30 calves per group, blood samples were taken to evaluate the serum antioxidant capacity and the haemoglobin level at d_0 and d_{90}.

In terms of zootechnical performances, no statistically significant differences were found. The incidence of pulmonary lesions was comparable in the two groups. No statistically significant differences were found also in terms of haemoglobin levels and oxidative stress. ROMs and OXY levels were similar between groups (284.92 in TREAT vs 365.23 in CON for ROMs and 237.90 HClO/mL in TREAT *vs* 228.45 HClO/mL in CON for OXY). In terms of antibiotic use, the control group received both more mass (17 in TREAT vs 21 in CON) and individual treatments (140 calves treated in TREAT vs 300 in CON), with an average increase of the days on treatment per animal (44.85 in TREAT and 57.52 in CON).

The inclusion of *Yucca Schidigera* allowed a reduction of the antibiotics use, but did not affects growth performance and carcass characteristics.

KEY WORDS

Antimicrobial Resistance; Veal Calves; Natural Extract; *Yucca Schidigera*.

INTRODUCTION

Antimicrobial resistance is a global health concern. Globally, 700,000 people each year die from infection with antibiotic-resistant organisms (AROs), one third in children aged under 5 years[1] and it is projected to cause 10 million deaths per year by the year 2050. Also, infection caused by antibiotic resistant microorganism represent an increasingly important cost item in a state's health care budget, that is expected to reach 100 trillion of dollars in 2050[2].

The mechanisms besides the acquisition of resistance characteristics by microbes such as bacteria, is complex, and still unclear[3]. It is generally accepted that resistant bacteria are a result of selection pressure[4]. Suspected principal foci of selection pressure include misuse and abuse of antimicrobials in both human medicine and food-producing animals farming for treatment or prevention (in the form of prophylaxis or metaphylaxis) of disease[2,5]. In a meta-analysis of data from 901 studies from 2000 to 2018, the proportion of antibiotics with resistance higher than 50% increased from 0.15 to 0.41 in chickens, 0.13 to 0.34 in pigs, and 0.12 and 0.23 in cattle. The highest resistance rates were observed among antibiotics used most commonly to avoid the onset of pathologies that can worsened animal productivity and welfare (tetracyclines, sulphonamides etc.)[6]. Among the different zootechnical species there are many differences in terms of use of antibiotics, considering the specific intrinsic characteristics of both the animals reared and the management systems. Globally, swine and poultry farming accounted for the highest use of antibiotics, while cattle farming is in third position in terms of milligrams per population correction units (PCU) (172 mg/PCU, 148 mg/PCU and 45 mg/PCU respectively)[6]. Also, the physiological stage of life of

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the animal reared can determine a different use of antibiotics. Young animals are more susceptible to diseases than adults and also their ability to cope is impaired, because of the low immune functionality and low antibody production. Consequently, the quantities and number of antimicrobials treatments are higher in young animals[7,8,9]. In swine farming, 80% of the total treatments are administered before the tenth week of life, and the most common are oral mass treatments[7].

Also, in beef cattle farming the use of antibiotics increase during the earlier stages of life. A clear example is the situation in white veal calves farming. The incidence of treatments, especially mass treatments, is much higher than in the fattening of adult beef cattle, and 12% to 13% of the total treatment are mass treatment[10]. White veal calves face many challenges during the first period of life, including birth, transportation, mixing procedures, inappropriate management conditions and new housing environments[11]. Furthermore, all these challenges occur at an age at which the calf is immature and several physiological systems are still developing and not completely functional yet, such as the gastrointestinal tract (GIT), the thermoregulatory and the acquired immune systems[12]. The combination of the indicated challenges and the immature physiological systems of the calves may explain the high susceptibility of calves to infections, and the higher use of antibiotics especially for enteric diseases and Bovine Respiratory Disease (BRD). While the first can sharply increase the mortality rate in the first 180 days of life, BRD is the main cause of production losses and welfare issues, with an economical loss of about 35 to 180 €. Traditionally, to prevent those heavy losses, farmers use metaphylactic mass treatments at the arrival and also others when the season or the specific situation of a batch are critical[8,9].

This situation must change because of antimicrobial resistance. In fact, in white veal farms the incidence of resistant bacteria is between highest in the zootechnical sector, specifically in terms of *Methicillin-Resistant Staphylococcus Aureus* (LA-MRSA)[13]. Alternative solutions to antibiotics have to be found, that can ameliorate the natural immune response of the calves, allowing to reduce the antibiotic use without an impairment in animal welfare and productivity standards.

In the last few years, natural products such as oil and extracts, are increasingly being studied in animal nutrition, because of their specific functional properties. Those products contain a pool of different active compounds, such as flavonoids, glucosinolates and isoprene derivatives, that have had an antibiotics-like and antioxidant-like action in different studies, done either *in vivo* or *in vitro*[14]. The extract from *Yucca Schidigera* has been proven to be rich in functional compounds such as ellagic acid, quercetin, tannins and cinnamic acids, saponins and other phenolic compounds, such as *yuccaols*[15]. The main biological activities are related to yuccaols and saponins[16,17].

The *Yucca Schidigera* extracts have shown positive results in zootechnical applications in food-producing animals, monogastric and ruminants, both in terms of production performances and immune functionality[18,19,20]. In terms of cattle farming, the inclusion of Yucca Schidigera extracts has led to a better immune functionality *in vivo*[21] (Mowat et al., 1999), mainly due to a better development of the immune response and a higher proliferation of the immune cells[22] and to a modulatory activity on the inflammatory reaction, that limits its negative effects through an action on specific transcription factors[23]. Furthermore, *Yucca's* saponins can have a modulating action on gut microflora. The combination of those factors can lead to better growth performance, especially in the early stages of life and in stressful conditions, as highlighted by de Sousa et al. (2019)[20]. Furthermore, saponins from *Yucca Schidigera* have shown a potential antimicrobial effect against *Escherichia coli*, mainly due to a lytic action on the bacterial membrane[24], and a potential anticoccidial effect in calves, reducing the excretion of oocysts[25].

The purpose of this study was to evaluate the potential effect of *Yucca Schidigera* extracts in the milk replacer of white veal calves on health status, immune functionality, antibiotic use and zootechnical performances, with the overall aim to reduce the use of antibiotics without welfare and productivity issues.

MATERIALS AND METHODS

Animals, housing and experimental groups

The study took place in a veal calves fattening facility, located in the Piemonte region.

For the purpose of the study, calves were housed in two different barns, filled completely in one day each (17th February and 8th March). The trial started at 17th of February 2019 and end at 2th October 2019, following the entire fattening period.

As the legislation reports, for the first 8 weeks of ages, all the calves were housed in single cage. For the other 8 months, calves were housed in group pens, with six calves each[26].

In the trial, 1015 male calves were involved, mostly Holstein-Friesian and some crossbreeds. In order to avoid the bias due to the batch, at the arrival, the animals were blocked by body weight, and assigned to the two experimental groups (Table 1), following the output of a numerical randomized procedure of Excel. In order to avoid a possible influence of the housing environment, both barns were divided into control and treatment. The two experimental group was: i) Control (CON), milk replacer without any nutraceutical inclusions; ii) Treatment (TREAT), milk replacer with the inclusion of two different nutraceutical supports, based on *Yucca Schidigera* extracts (Table 2). The first (MiniMix START 0-60) was specially formulated to promote the immune function and antioxidant status of the calves, while the second (MiniMix PROGRESS CB) to counteract the digestive disorders. The first nutraceutical support was administered for the first 60 days of fattening (15 g/head/day), while the second one only in case of digestive issues, for only 5-7 days (20 g/head/day) from the 40th day of life. The feeding plan and the characteristics of the milk replacers used were the same for the two experimental group. During the hole fattening period three different milk replacer were used, to better satisfy the specific nutritional requirements in the different physiological phases, accordingly to the NRC (2001)[27]. The *Yucca Schidigera* extracts was mixed directly into the milk replacer. Milk was offered in two equal meals daily at 09:00 and 17:00. All the calves were bucket-fed the milk-replacer diet. During the hole fattening period all the calves received the same type and amounts of solid feed. All the calves had free access to the water.

In terms of sanitary management protocol, at restocking both the groups receive oxytetracycline for eleven days as a preventive mass treatment. Calves from the control group received also colistin, as another mass treatment at restocking, for five

Table 1 - Experimental groups: CON vs TREAT.

	CON	TREAT
	Characteristics	
n of calves	490	525
Average arrival weight, kg	47.8	47.6
Average arrival age, d	24.1	22.8
	Restocking sanitary protocol	
d_0 to d_5	Mass feed treatment: Oxytetracycline + Colistin	Mass feed treatment: Oxytetracycline
	Nutraceutical support	
d_0 to d_{60}	-	15 g/head/day of MiniMix Start 0-60
In case of digestive disorders	-	20 g/head/day of MiniMix Progress CB

Table 2 - Characteristics of the two nutraceutical premixes.

	MiniMix START 0-60	MiniMix PROGRESS CB
Components	*Yucca Schidigera* extracts and dehydrated yeast culture of *Saccharomyces cerevisiae*	*Yucca Schidigera* extracts and dehydrated yeast culture of *Saccharomyces cerevisiae*
Analytical values, % DM		
Crude protein	20,00	11,00
Crude fats	1,50	0,90
Ash	11,00	23,00
Cellulose	23,00	12,50
Mg	0,75	1,80
Methionine	0,30	0,15
Doses (g/head/day)	15	20

days, as reported in Table 1. After restocking the standard sanitary management protocols didn't differ between the two groups.

Parameters

Indicators of the immune functionality were analysed in combination with data about health status, antimicrobials use and zootechnical parameters, observed both at the farm level and at slaughterhouse.

Production performances, health status and antibiotic use

The animals were monitored on a daily basis by the farm's veterinary staff. Mortality and morbidity were recorded daily with also the relative causes. The veterinary treatments administered in both groups were recorded on the farm register, divided in individual and mass treatments. The quantities of antibiotics administered were calculated, based not only on the number of animals involved, but also on the duration of each intervention (days on treatment).

At slaughterhouse, the carcass weight, fattening and conformation score and colour indicators were recorded. The average daily gain (ADG, kg/head/day of weight) was then calculated. Furthermore, during the *post mortem* inspection, the pulmonary scores were done, according to Leruste et al. (2012)[28]. Both the left and right lungs were examined (cranial and caudal lobe for the left lung and cranial, caudal and intermediate lobe for the right lung). The presence of lung lesions was evaluated, in terms of both number and also severity and extension, with a 0 to 3 evaluation score, reported in Table 3.

Evaluation of oxidative stress levels (TEST ROMs and OXY)

On 30 calves per group, at d_0 and d_{90}, blood samples were taken to determine the antioxidant status.

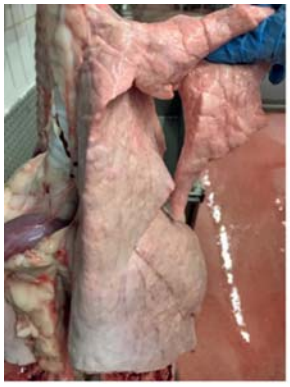
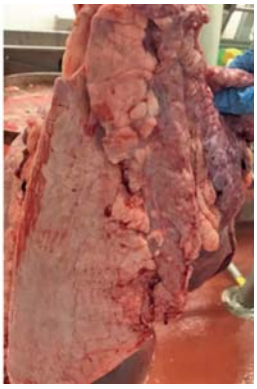
Blood samples were collected from the jugular vein using tubes with EDTA just before morning feeding, and the tubes were immediately placed on ice. The collected samples were centrifuged at 2500 × g for 15 min at 4 °C, and thereafter plasma samples were stored at -20 °C until analysis.

Serum was analysed for serum antioxidant status, through the oxygen reactive metabolites (ROMs) test, and for antioxidant capacity through the OXY-adsorbent test.

The ROMs test was done according to Iorio et al. (2003)[29]. The ROMs test allows to determine the blood concentration of the hydroperoxides (ROOH), substances belonging to the wide class of the so-called reactive oxygen metabolites (Reactive Oxygen Metabolites, ROMs), markers of the tissue damage generated by the peroxidation of lipids, amino acids, proteins, and nucleic acids. In this test, hydroperoxides, after reacting with a suitably buffered chromogen (N, N-diethyl-para-phenyl diamin), develop a coloured derivative, which is detected photometrically. The concentration of hydroperoxides, directly proportional to the intensity of the colour, it is expressed in Carratelli Units (1 U CARR= 0.08 mg hydrogen peroxide / dL).

The OXY-adsorbent test was done according to Iorio et al.

Table 3 - Lungs evaluation.

Score*	0	1	2	3
Images				
Notes	Healthy lungs, light pink-orange colour	Lungs with a small lesion grey-red coloured	Lungs with one big or several small lesions grey-red coloured, with total surface smaller than a lobe	Lungs with lesion grey-red coloured, with a total surface of at least a lobe and/or presence of abscesses

*Evaluation scale based on Leruste et al., (2012)

(2003)[29]. This test evaluates the capacity of the serum samples to cope with a massive oxidant attack, inducted *in vitro* by a hypochlorous acid solution. Unreacted HClO radicals further react with the chromogen solution of N, N-diethyl-p-phenylendiamine and form a colored complex, which is measured at 505 nm. The results of the test are expressed as mol HClO/mL. The highest are the results, the better is the defence ability of the plasma.

Statistical analysis

Statistical analyses were performed using SAS 9.3 (2010; SAS Institute Inc., Cary, NC, USA). The data obtained were subjected to analysis of variance, using the General Linear Model procedure of SAS.

RESULTS

Zootechnical, health and productive parameters

The zootechnical and productive data, registered at the farm level and at slaughterhouse, are summarized in Table 4. For these parameters, no statistically significant differences were found. Mortality and incidence of lighter carcasses, weighing less than

Table 4 - Zootechnical performances.

	N°	Mortality, % (n)	ADG, kg/d	Carcass Weight, kg	Losses (<110 kg), %	Color L,	a,	b
Control	490	2.86 (14)	1.270	170.23	1.26	41.71	10.01	4.26
Treatment	525	4.76 (25)	1.273	169.17	2.00	42.14	9.77	4.21
P	ns							

Table 5 - Results of the pulmonary score evaluation.

	Pulmonary Score, % (n)			
	Score 1	Score 2	Score 3	Score 4
Control	43.61 (181)	19.04 (79)	23.61 (98)	13.73 (57)
Treatment	46.88 (210)	15.40 (69)	23.88 (107)	13.84 (62)
P	*ns*			

Table 6 - Evaluation of the oxidative stress at day 0 and 90 and evaluation of the haemoglobin level during the hole fattening period.

	day	Control	Treatment	P
ROMs, U/Carr	d0	259.00	258.69	
	d90	365.23	284.91	
OXY, µmol HClO/mL	d0	316.37	303.81	*ns*
	d90	228.45	237.90	
Hb, g/dL	d0	9.29	9.14	
	d90	8.92	8.92	

Table 7 - Individual treatment.

	Stable B1		Stable C1		Weighed Total (n° calves* days of treatment)	
	C	T	C	T	C	T
Total calves per group	265	253	225	272	490	525
N° calves treated individually	152	45	148	95	931	425
Total days of treatment					19.046	20.761
Average treatment days for each calf					38.8	39.5

110 kg, were similar between groups (respectively, 2.86% *vs* 4.76% in TREAT and 1.26% *vs* 2% in CON). No differences were also found for ADG, final carcass weight and colorimetric characteristics.

The results of the pulmonary inspection are summarized in Table 5. Also, for pulmonary score there were no statistically significant differences between the two groups. The higher number of severe lesions (score 2 and 3) highlighted in the TREAT group was only a function of the greater numerosity of calves in this group (525 in TREAT *vs* 490 in CON). In fact, in percentage terms, the distribution of observations in the 4 different classes was similar between the two groups.

Evaluation of oxidative stress levels (TEST ROMs and OXY)

In Table 6 are reported the data obtained from the ROMs and OXY analysis at d_0 and d_{90}.

No statistically significant differences were found also for those parameters. Indeed, the inclusion of the two nutraceutical nutrients did not affect the antioxidant capacity of calves. In both groups at d_{90} the level of ROMs (284 U/Carr in TREAT *vs* 365 U/Carr in CON), and OXY (237.90 HClO/mL in TREAT *vs* 228.45 HClO/mL in CON) were similar.

Antimicrobials use: type of treatment and days on treatment

In Table 7 and 8 are reported all the treatments done in both stable and in both groups, divided for "individual" (Table 7) or "mass" treatment (Table 8). Also, the number of calves treated and the days on treatment are reported, to better quantify the use of antibiotics in both groups.

As visible in Table 7, the number of calves treated individually in the CON group was higher (152 and 148 in CON *vs* 45 e 95 in TREAT), but because calves treated in TREAT group undergone to a longer treatment period, the total days on individual treatment didn't differ between the two groups.

As visible in Table 8, calves from the CON group received two more mass treatment in each barn, one (colistine) as a part of

Table 8 - Mass treatment, total treatment and days on treatment per group.

	Stable B1		Stable C1		Weighed Total (n° calves* days of treatment)	
	C	T	C	T	C	T
Total calves per group	265	253	225	272	490	525
Mass treatment					27.255	23.119
15-25/02	Oxytetracicline		-	-	2.915	2.783
15-19/02	Colistine	-	-	-	1.325	-
20-25/02	Sulf. + Trimethoprim	-	-	-	1.590	-
22/02	Tulathromycin	Tildipirosin	-	-	265	253
02-04/03	Oxytetracicline	-	-	-	795	-
08-15/03	-	-	Oxytetracicline		1.800	2.176
08-11/03	-	-	Colistine	-	900	-
11-15/03	-	-	Sulf. + Trimethoprim	-	1.125	-
16/03	-		Tulathromycin	Tildipirosin	225	272
16-20/03	Doxicicline		-	-	1.325	1.265
27-30/03	-	-	Oxytetracicline		900	1.088
05-08/04	-	-	Doxicicline		900	1.088
24-28/04	Amoxicillin		-	-	1.325	1.265
11-16/05	-	-	Amoxicillin		1.350	1.632
26-30/05	Amoxicillin		-	-	1.325	1.265
09-13/05	-	-	Amoxicillin		1.125	1.360
18-23/06	Oxytetracicline		Oxytetracicline		2.940	3.150
09-13/07	Oxytetracicline		Oxytetracicline		2.450	2.625
02-06/08	Amoxicillin		-	-	1.325	1.265
26-31/08	-	-	Amoxicillin		1.350	1.632
N° calves treated individually	152	45	148	95	931	425
Total days on treatment					28.186	23.544
Average days on treatment for each calf					57,52	44,85

the standard sanitary protocol and one as a result of an impairment in the health status. CON calves resulted to be treated four more times than the TREAT group. Also, the total days on treatment was affected, even when the individual and mass treatment were considered together, resulting in higher total and average days on treatment in the CON group (Table 8).

DISCUSSION

The spread of antimicrobial resistance and its implication in human health has led the zootechnical producers to rethink about their standard management and sanitary practices, to find out how to reduce this phenomenon. The white veal calf farming is also deeply involved in this process. Traditionally, a high use of orally administered mass treatments is done, especially for preventive purposes. Those type of treatments are considered the main cause of the growth of strains of resistant bacteria.

This research was conducted to verify the effect of an inclusion of *Yucca Schidigera* extracts on growth, health, immune function of white veal calves and on the average use of antibiotics, specifically mass treatments.

In bibliography it was proved that *Yucca Schidigera* can have many important biological implications, especially because of its content in saponins and resveratrol. Besides having a strong immunostimulatory capacity, it also promotes digestive function, both at ruminal and intestinal levels[18].

Considering the antioxidant status, as visible in Table 5, it seems that the administration of *Yucca Schidigera* extract did not significantly alleviate the oxidative stress, even if calves from the TREAT group showed a slightly lower values for ROM analyses and, on the other hand, slightly higher values for µmols of HClO/mL, factors indicating a better reaction of the antioxidant systems. However, other studies that involved *Yucca Schidigera* in animal nutrition and in *in vitro* tests, showed positive effects on the oxidative status of treated animals. Resveratrols and other specific polyphenols, called "yuccaols", have been shown to have a remarkable antioxidant effect, often even superior to the reference antioxidants, both *in vivo* and *in vitro* tests (Test Teac)[15,17]. Unfortunately, the difference found in this study was only a numerical difference and it wasn't statistically significative.

Several studies have also shown a potential stimulating effect of saponins on immune cells and on the production of antibodies[22]. Neither there was a positive effect of yucca administration in terms of pulmonary score.

CONCLUSIONS

Considering the result obtained in this trial, it can be concluded that the inclusion of a nutritional support based on natural extracts as *Yucca Schidigera*, is not sufficient to obtain a real and significant reduction in the consumption of antibiotics.

The continuous spread of antimicrobial resistance pushes the zootechnical producers and technicians to study and search new strategies to reduce the use of antibiotics, without at the same time affecting animal welfare and productivity. Natural extracts and essential oils surely will have many applications in the future, because of their multiple biological functions. But, as underlined in this research, this is not enough to obtain a real improvement in antibiotic reduction. To achieve this result is necessary to rethink about the hole farming process, not only the nutritional integration. In fact, there are many different aspects that can impact on both animal immune function and pathogens circulation in white veal calves farming. Creating an "integrated management system" that consider all those aspects that can affect animal strengthens and welfare can be the only way to effectively reduce the use of antibiotics. All those factors can be summarized in the more general concept of "animal welfare". In fact, a lower animal welfare can cause both a higher spread of pathogens and a stronger incidence of disease. It could be explained considering the effect of stress on immune function and production parameters. A lower welfare exposes the animals to a higher stress levels, that can have a detrimental effect on the immune system, lowering their ability to cope with pathogens.

On one hand, it is necessary to individuate and limit all the risk factors that can lead to a higher microbial circulation, or to a less immune functionality. On the other side, it is important to find out how animal welfare and strengthens may be improved and act on those aspects.

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