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Influence of spray-dried rumen fluid supplementation on performance, blood metabolites and cytokines in suckling Holstein calves

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*Rumen fluid from slaughtered animals is one of the wastes of slaughterhouses released to the environment that, due to its high nitrogen and phosphorus contents, can lead to soil and groundwater pollution. Meanwhile, it contains ruminal microbes and some bioactive compounds such as enzymes, minerals, vitamins and organic acids. This study was designed to examine the potential of rumen fluid as a feed additive. Therefore, the effects of spray-dried rumen fluid (**SDR**) with 1% maltodextrin on the performance, blood metabolites and some cytokines of sucking dairy calves during the pre-weaning phase were investigated. Forty male Holstein calves, with a mean weight of 39.4 ± 3.7 kg and 7 ± 1 days old, were randomly assigned to four groups (*n *= 10 calves per group) in a completely randomized design. Experimental treatments were: control diet with no additive (**CON**); control diet with 0.5 g/day of SDR (**SDR0.5**); control diet with 1 g/day of SDR (**SDR1**); and control diet with 1.5 g/day of SDR (**SDR1.5**). Daily feed intake and average daily gain of calves were not affected by feeding SDR as a feed additive. Cholesterol concentration was significantly affected by the 20th and 40th days of the experiment and decreased linearly by increasing SDR feeding level. Levels of liver enzymes, including aspartate aminotransferase and alanine aminotransferase, in the blood decreased by feeding SDR at day 40 of the experiment. Serum concentration of interleukin-6 at day 20 was not affected by dried rumen fluid feeding, whereas at day 40, a significant effect was observed among experimental treatments. The lowest value was recorded for SDR1.5 v. control calves. At day 20, the serum concentration of interferon-γ was influenced by supplementing SDR, and the highest value was recorded for SDR1.5 calves. The inclusion of SDR with 1% maltodextrin in suckling dairy calves had beneficial effects on the stimulation of calves' immune system.*

Keywords: abattoir's wastes, feed additive, immune system, interleukins, interferon

Implications

Rumen fluid from slaughtered animals is one of the wastes of slaughterhouses released to the environment that, due to its high nitrogen and phosphorus contents, can lead to soil and groundwater pollution. Meanwhile, it contains ruminal microbes and some bioactive compounds such as enzymes, minerals, vitamins and organic acids. This study was conducted to examine the potential of spray-dried rumen fluid as a feed additive for suckling dairy calves. Results showed that spray-dried rumen fluid had some positive effects on the immune parameters of calves, confirming the benefits of rumen fluid application as a feed additive in animal nutrition.

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Introduction

Everyday millions of ruminant animals are slaughtered throughout the world and their rumen fluids disposed to the environment, causing pollution to the soil and groundwaters (Rezai Sarteshnizi *et al.*, 2018a). By appropriate processing for use as an ingredient in animal feeds, it will not only decrease the pollution but also provide new opportunities in animal feeding practices (Cherdthong and Wanapat, 2013).

The amount of rumen content varies with the type of ruminant animal and body weight, and an average of 10 kg was reported in small, and 40 kg in large ruminant animals (Afazeli *et al.*, 2014; Abdeshahian *et al.*, 2016). It includes fermented and unfermented solid feed materials, ruminal

microorganisms and end-products of microbial activity in a fluid medium called the rumen fluid. The rumen fluid is rich in different nutrients such as microbial proteins, amino acids, volatile fatty acids, vitamins, minerals (Elfaki and Abdelatti, 2018) and diverse enzymes (Rezai Sarteshnizi *et al.*, 2018a) without any anti-physiological factors (Okpanachi *et al.*, 2010). It also contains several microorganisms, including bacteria, protozoa and fungi (Koike and Kobayashi, 2001; Calabro *et al.*, 2012).

In an early work, the positive effects of ruminal fluid inoculation on dairy calves were indicated (Pounden and Hibbs, 1949). Muscato *et al.* (2002), in a promising work, fed different ruminal fluid preparations, including fresh rumen fluid, autoclaved rumen fluid and centrifuged rumen fluid, to suckling calves and observed positive effects on weight gain of dairy calves and diarrhoea incidence. They proposed that rumen fluid could be given orally, mixed with milk. They stated that rumen fluid contains naturally occurring non-pathogenic bacteria whose effects on weight gain and diarrhoea incidence remain even if autoclaved. Therefore, based on its effects after autoclaving, it did not act as a probiotic (Muscato *et al.*, 2002). The ruminal bacteria have a thick coating of bacterial polysaccharides (Costerton *et al.*, 1974), but the impact of this material on the ruminant immune system has mostly been ignored. These polysaccharides may act as a trigger for antibody production (Muscato *et al.*, 2002). Furthermore, polysaccharides from ruminal bacteria may (1) act as an adjuvant to enhance the potency of other antigens, (2) induce macrophages to release cytokines that affect the differentiation of mammalian cells and (3) circumvent the normal cascade of immune stimulation to cause an anergy commonly called oral tolerance (Tizard, 1996; Roitt *et al.*, 1998).

In practice, the moisture content of the entire rumen content collected in slaughterhouses is considered one of the barriers that need a proper solution (Abouheif *et al.*, 1999). For the production of fixed and preserved biologically active compounds, spray drying has been proposed (Terebiznik *et al.*, 1997). Spray drying is a method for transforming a liquid into powder form (Bajsic and Kranjcevic, 2001). It is the most prevalent technique used in pharmaceutical and milk industries for drying different substrates such as milk, whey, antibiotics, vitamins and enzymes. (De Vos *et al.*, 2010). Short drying time and relatively low temperature have made spray drying a useful method for drying sensitive materials (Namaldi *et al.*, 2006). Drying changes a liquid to its solid state that allows easier handling, storage, transportation and better mixing in feed or food formulations when small amounts have to be mixed (Shahidi and Han, 1993; Tan *et al.*, 2005).

Some materials are used in spray drying as encapsulated materials to protect the biologic molecules from heating. These materials include carbohydrates (starch, maltodextrin and dextrose), gums (arabic, acacia, alginate, chitosan) and proteins (milk or whey proteins, gelatine), which are referred to as hydrocolloids (Gouin, 2004; Krajewska, 2004; Gharsallaoui *et al.*, 2007). Many studies have shown the protective effects of binding agents in reducing heat stress of spray drying (Maury *et al.*, 2005; Jalalipour *et al.*, 2008). In a recent work, we examined the efficacy of different hydrocolloids on drying rumen fluid by spray drying and found maltodextrin to be an efficient material (Rezai Sarteshnizi *et al.*, 2018b).

Therefore, based on the diverse and biologically active compounds in the rumen fluid and lack of enough researches focusing on its application as a feed additive, the aim of this study was to evaluate dried rumen fluid with maltodextrin as a feed additive on the performance, blood parameters and immune system of suckling dairy calves.

Material and methods

Spray drying the rumen fluid

Rumen contents were collected from a slaughterhouse (Ardabil Industrial Meat Complex, Ardabil, Iran) and transferred to the laboratory in pre-warmed containers. A laboratory blender under constant CO_2 purging was used to obtain a homogeneous mixture. After that, the rumen content was filtered through a four-layer cheesecloth to separate rumen fluid from rumen solid materials. The collected rumen fluid was spray-dried after the addition of maltodextrin (Sigma-Aldrich, CAS number, 9050-36-6; Germany) as an excipient hydrocolloid in 1% (w/v) (Rezai Sarteshnizi *et al.*, 2018b). A semi-industrial-scale spray dryer (Maham Sanat Company, Sd2 Spray Dryer, Iran) was used with an inlet and outlet air temperature of 135°C and 65°C, respectively, fluid flow rate of 0.6 l/h and air flow rate of 200 m^3/h. After drying, the obtained powder was kept in two-layer polyester bags in a refrigerator at 5°C until use.

Chemical composition and enzyme activity of spray-dried rumen fluid

The analysis of spray-dried samples was done for DM (method number 930.15), crude protein (**CP**; Kjeldahl N × 6.25, method number 984.13), ether extract (**EE**, method number 920.39) and ash (method number 924.05) by AOAC (1997) methods. Based on laboratory results, dried ruminal fluid with 1% maltodextrin consisted of 93.80% DM, 12.27% CP, 2.60% EE and 19.6% ash.

For assessing the extent of heat effect from spray drying on rumen fluid and its residual biological activity after spray drying, some enzyme activities of dried rumen fluid were determined. For this purpose, the rumen fluid was reconstituted by dissolving 1 g of dried rumen fluid in 100 ml of 0.1 M phosphate buffer, pH 6.8 (buffer A) and analysed for the main polysaccharide enzyme activities, including carboxymethyl cellulase activity (CMCase), microcrystalline cellulase activity (Avicellase), amylase, and filter papers activity (Ftpase) (Agarwal *et al.*, 2000; Rezai Sarteshnizi *et al.*, 2018b). To determine CMCase activity, 0.5 ml of the reconstituted rumen fluid was added to 1 ml buffer A and 0.5 ml carboxymethyl cellulose solution (1% w/v in distilled water) and mixed thoroughly. To determine Avicellase activity, 1 ml of the reconstituted solution was mixed with 1 ml

microcrystalline cellulose (Avicel; Sigma-Aldrich, CAS number, 9004-34-6, Germany) solution (1% w/v in buffer A) and 1 ml buffer A. The mixtures were incubated for 60 min at 39°C. To measure amylase activity, 0.25 ml of the reconstituted rumen was mixed with 0.5 ml buffer A and 0.25 ml of 1% (w/v in distilled water) starch (Sigma-Aldrich, CAS number, 9005-25-8, Germany) solution. Thereafter, incubation was done for 30 min at 39°C. For determining Ftpase activity, 1 ml of the reconstituted solution, 1 ml distilled water and 1 ml buffer A were mixed and added to 50 mg Whatman filter paper strips (No. 1); the mixture was incubated at 39°C for 1 h. After incubation, 3 ml of 10% dinitrosalycylic acid solution (w/v in 2% sodium hydroxide solution) was added and incubated for a further 10 min at 100°C in a water bath. After adding 1 ml of 40% (w/v in distilled water) Rochelle salt solution, the mixtures were kept under tap water for cooling, and absorbance was read at 575 nm (UNICO 2100 spectrophotometer, Unico, USA). The absorbance was corrected for the medium of the mixture using a blank sample that includes all materials except spray-dried rumen fluid (**SDR**). Dinitrosalicylic acid was used to determine the contents of reducing sugars as described by Miller (1959). Enzyme activities were expressed as unit per ml (U/ml), that is, each unit is *m* mole of reducing sugars released per minute per millilitre of enzyme-containing solution under assay conditions. Because the reaction between glucose and dinitrosalicylic acid produced a red dye, standard glucose solutions (0, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 μmol of glucose) were used to quantify the glucose released from the mixtures after incubation. A standard calibration curve (plotting absorbance against standard glucose concentration) was used to determine the amount of glucose released in each tube.

Animals and treatments

Forty male Holstein dairy calves (average birth weight 39.4 ± 3.7 kg; age 7 ± 1 days; from Moghan Agro-Industrial and Animal Husbandry, Pars Abad, Ardabil province, Iran; on July and August 2018) were used to determine the effects of SDR with maltodextrin on the performance, blood metabolites and some cytokine concentrations of calves. Experimental treatments were: (1) control diet with no additive (**CON**); (2) control diet with 0.5 g/day of SDR (**SDR0.5**); (3) control diet with 1 g/day of SDR (**SDR1**); and (4) control diet with 1.5 g/day of SDR (**SDR1.5**). SDR was top-dressed on the starter feed and mixed thoroughly once a day on each morning from day 7 (from second week of life) until the end of experiment. Calves were assigned randomly among the groups based on their age and birth weight to get similar average weight and age among the treatments. Colostrum was fed from a nipple pail until 3 days of age and thereafter with 4 kg of whole milk per day in two meals for the first 2 weeks, 6 kg/day in two meals for third and fourth weeks, 4 kg/day for fifth week in two meals; and 2 kg/day in one meal for sixth week until weaning. Milk feeding was done twice a day (0800 and 1800 h) by bucket, and the entirety of offered milk was consumed by all the calves. The starter diet and water were offered *ad libitum* from day 7 of life, and chopped alfalfa at the rate 10% was added to the ration of calves from the 20th day of birth. A bucket fixed to the door of each pen was used for feeding solid feeds. The ingredients and chemical composition of the starter diet, alfalfa hay and milk are shown in Table 1. The calves were kept in individual pens (1 × 2.5 m) with straw bedding that was removed and cleaned daily. All animal procedures were based on the guidelines of the Iranian Council of Animal Care (1995).

Table 1 *Ingredients and chemical composition of starter feed, alfalfa hay and milk (DM basis) fed to calves*

Item	Starter	Alfalfa hay	Milk
Ingredient (%)			
Corn	42.50	–	–
Barley	12.00	–	–
Wheat bran	5.00	–	–
Soybean meal	37.60	–	–
Salt	0.40	–	–
Oyster shell ground	1.00	–	–
Mineral and vitamin premix[1]	1.00	–	–
Di-calcium phosphate	0.50	–	–
Chemical analysis (%)			
DM	89.70	88.90	12.46
NE_m (Mcal/kg)	2.20	–	–
NE_g (Mcal/kg)	1.67	–	–
Crude protein	18.70	15.80	3.26
Ether extract	2.26	2.04	3.75
Neutral detergent fibre	16.25	53.73	–
Acid detergent fibre	7.31	38.75	–
Calcium	0.62	1.39	–
Phosphorus	0.50	0.22	–

NE_m = net energy for maintenance; NE_g = net energy for growth.
[1]Vitamin premix provided per kilogram of diet: vitamin A, 200 000 IU; vitamin B, 300 000 IU; vitamin E, 10 000 IU; vitamin K, 2 mg; antioxidant 1000 mg/kg. Mineral premix provided per kilogram of diet: Cu, 3300 mg/kg; Fe, 100 mg; Zn, 16 500 mg/kg; Mn, 9000 mg; I, 120 mg/kg; Co, 90 mg/kg; Se, 90 mg/kg.

Throughout the experiment, the calves were weighed on days 10, 20, 30 and 40 in the morning individually without prior deprivation of feed and water, and weight change was calculated by subtraction method. During the experiment, feed intake was measured daily by the difference between feed offered and feed refused.

Faecal consistency was determined in 10-day intervals, according to McGuirk (2008). So, a score of 0 was assigned for faeces with normal consistency, 1 for semi-formed or pasty faeces, 2 for faeces with loose but enough consistency to remain on bedding, and 3 was given to watery faeces that sift through the bedding material. On test day, two trained practitioners scored the faeces of calves, and their average was used for further analysis.

Blood samples were taken from the jugular vein, on days 20 and 40 of the experiment 3 to 4 h after morning feeding, into two separate tubes (one with heparin and the other with no anticoagulant). Plasma and serum were collected by centrifuging the blood samples at 3500×**g** for 15 min at 4°C. The samples were kept at −20°C until analysis. The obtained plasma samples were analysed for glucose, cholesterol, total

protein, albumin and globulin, aspartate aminotransferase and alanine aminotransferase colorimetrically using commercial kits (Pars Azmoon Co, Tehran, Iran). Recent data have indicated that probiotic (Barnes *et al.*, 2007; Sun *et al.*, 2010) and prebiotic (Szymanska-Czerwinska *et al.*, 2009; Capitan-Canadas *et al.*, 2014) feed additives may have immunomodulation effect, modifying the immune response of T-helper cells 1 and 2, increasing blood cytokines and thereby enhancing the immune function of the animal. Based on these data, serum samples also were analysed to determine the concentrations of interleukin-6 (**IL-6**), IL-10 and interferon-γ (**IFN-γ**) with the Bovine ELISA Kit (intra-assay CV <10; inter-assay CV <12; Hangzhou Eastbiopharm, China) according to the manufacturer's instructions.

No severe disease incidence occurred during the experiment. Only four diarrhoea cases (1 in control, 2 in SDR0.5 and 1 in SDR1.5) were observed. No mortality was observed among the experimental groups.

Data analysis

Repeated measurement analysis was done for the performance data using the *Mixed* procedure of SAS statistical software. Treatment, time and their interaction (treatment × time) were used as fixed effects, and individual calves were used as random effect. Because the interaction of treatment × time had not significant effect on the results, based on the repeated measurement analysis, it is not reported in the tables. The time effect was significant for all performance data ($P < 0.01$).

Data of faecal consistency and blood samples were analysed by the GLM procedure of SAS statistical software (SAS, 2003) as a completely randomized design according to the following model:

$$Y_{ij} = \mu + T_i + b\left(x_{ij} + \overline{X}\right) + E_{ij}$$

where Y_{ij} is a dependent variable; μ is overall mean; T_i is the effect of dried rumen fluid (treatment); b is the regression coefficient of Y on initial body weight (X); and E_{ij} is the residual error.

The contrast statement of SAS was used to evaluate the linear and quadratic effects of treatment. Least squares means were determined for each treatment, and the Tukey–Kramer test of SAS was selected to declare differences among treatments. Significant differences were declared at $P \leq 0.05$.

Results

Enzyme activity of spray-dried rumen fluid

The determination of some enzyme activities of SDR was done to evaluate the extent of heat effects of spray drying on rumen fluid and its residual biological activity after spray drying. Based on laboratory analysis, SDR had 73.58 U/ml for CMCase activity, 28.95 U/ml for Avicellase activity, 725.28 U/ml for amylase activity, and 88.03 U/ml for Ftpase activity. The obtained data are in correspondence with the amounts reported earlier for extracellular enzyme activities in rumen fluid (Agarwal *et al.*, 2000; Azizi-Shotorkhoft *et al.*, 2018; Rezai Sarteshnizi *et al.*, 2018b). Rezai Sarteshnizi *et al.* (2018b) showed 92%, 73%, 84% and 95% CMCase, Avicellase, amylase and Ftpase activities, respectively, in rumen fluid after spray drying with 1% maltidexterin as excipient.

Performance

Daily feed intake was not affected by SDR. Some numerical, but not significant, decreases were noticed when increasing the level of feeding SDR. No adverse effect from feeding SDR was observed on the average daily gain of calves, and there were no differences among the treatments. Feeding increased doses of SDR did not affect the feed conversion ratio (**FCR**) of calves during the experiment (Table 2).

Among the four periods tested, there was no statistical difference among the treatments on faecal scores (Table 2).

Blood metabolites

Glucose, urea, albumin, triglyceride and total protein concentrations did not show any affect from SDR feeding. However, cholesterol concentration was affected on the 20th and 40th days of the experiment, and decreased by increasing SDR feeding level ($P < 0.05$). However, calves fed 1.5 g SDR per day had lower cholesterol concentrations compared to control calves ($P < 0.05$). Triglyceride concentration increased quadratically when increasing SDF feeding level ($P < 0.05$). The level of liver enzymes, including aspartate aminotransferase (**AST**) and alanine aminotransferase (**ALT**), in the blood decreased linearly by increasing feeding doses of SDR ($P < 0.05$). The highest level of SDR, at 1.5 g/day, resulted in lower enzyme levels in comparison to controls ($P < 0.05$). At day 20, ALT levels were not affected by feeding different levels of SDR (Table 3).

Concentrations of cytokines

The serum concentrations of IL-6 and IL-10 at day 20 were not affected by SDR feeding, whereas at day 40, significant effects were observed among experimental treatments. Increasing doses of SDR decreased the concentrations of IL-6 and IL-10 linearly ($P < 0.05$). The lowest ($P < 0.05$) values was recorded for the SDR1.5 group, and the highest ($P < 0.05$) IL-6 concentration was measured for the SDR0.5 group. At day 20, the serum concentration of INF-γ was influenced by supplementing SDR, and the highest value was recorded for the SDR1.5 group. No significant differences were observed for the INF-γ concentration at the 40th day of the experiment (Table 4).

Discussion

The use of rumen contents as fertilizer (Tritt and Schuchardt, 1992) or for biogas production (Afazeli *et al.*, 2014) has been proposed for managing abattoir's rumen wastes. In addition, some efforts have focused on the use of rumen solid contents as a feed in animal nutrition (Abouheif *et al.*, 1999; Cherdthong *et al.*, 2014). Positive effects from

Table 2 *Effect of different levels of SDR[1] on growth performance and faecal consistency in calves*

Item	CON	SDR0.5	SDR1	SDR1.5	SEM	Main effect	Contrasts	
							Linear	Quadratic
Birth weight (kg)	39.38	39.25	39.44	39.50	1.30	0.99	0.92	0.94
Final weight (kg)	65.82	65.41	65.51	65.35	0.82	0.96	0.65	0.80
Feed intake (g/d)								
10 days	125.0	118.8	123.7	107.5	13.47	0.78	0.78	0.71
20 days	213.7	208.7	204.4	190.6	18.01	0.82	0.56	0.87
30 days	397.1	380.0	378.8	366.3	23.31	0.82	0.71	0.96
40 days	548.4	529.4	531.3	512.2	18.33	0.58	0.70	0.99
Daily weight gain (g/d)								
10 days	254.0	242.9	249.3	256.3	15.68	0.93	0.98	0.56
20 days	327.0	320.2	312.1	323.8	18.96	0.95	0.87	0.70
30 days	428.9	422.2	414.3	421.3	21.45	0.97	0.85	0.85
40 days	587.6	589.5	590.8	583.4	19.13	0.99	0.96	0.93
FCR[2]	0.86	0.85	0.85	0.81	0.05	0.62	0.54	0.80
Faecal consistency								
Day 10	1.50	1.75	1.75	1.37	0.15	0.81	0.80	0.36
Day 20	1.75	1.43	1.62	1.87	0.14	0.76	0.68	0.35
Day 30	1.62	1.75	1.87	1.75	0.14	0.96	0.72	0.69

[1]Treatments: CON = control diet with no additive; SDR0.5 = control diet with 0.5 g/day of SDR; SDR1 = control diet with 1 g/day of SDR; SDR1.5 = control diet with 1.5 g/day of SDR.
[2]Feed conversion ratio.

Table 3 *Effect of different levels of SDR[1] on the blood metabolites of calves*

Item	CON	SDR0.5	SDR1	SDR1.5	SEM	Main effect	Contrasts	
							Linear	Quadratic
Glucose (mg/dl)								
Day 20	96.50	81.87	85.62	82.37	2.70	0.19	0.11	0.28
Day 40	60.87	52.37	52.37	52.00	2.20	0.42	0.18	0.36
Cholesterol (mg/dl)								
Day 20	119.75^{a}	101.00^{ab}	107.75^{ab}	95.00^{b}	3.50	0.067	0.02	0.64
Day 40	107.00^{a}	94.37^{ab}	101.62^{a}	87.00^{b}	2.59	0.028	0.01	0.83
Triglycerides (mg/dl)								
Day 20	13.62	19.37	23.62	16.87	1.47	0.09	0.26	0.03
Day 40	19.50	20.87	21.75	15.50	1.02	0.13	0.21	0.06
Urea (mg/dl)								
Day 20	22.12	22.12	23.87	22.25	0.92	0.78	0.55	0.49
Day 40	17.12	18.12	20.25	17.12	0.88	0.75	0.79	0.25
Albumin (g/dl)								
Day 20	2.71	2.75	2.86	2.75	0.03	0.28	0.35	0.29
Day 40	2.73	2.37	2.87	2.68	0.03	0.33	0.97	0.21
Total protein (g/dl)								
Day 20	6.78	6.63	6.62	6.62	0.09	0.92	0.57	0.70
Day 40	7.01	6.69	6.95	6.85	0.09	0.94	0.56	0.89
AST (U/ml)								
Day 20	38.75	38.50	33.62	32.12	1.33	0.17	0.03	0.77
Day 40	58.12^{a}	52.75^{ab}	47.32^{ab}	42.00^{b}	2.20	0.05	0.00	0.94
ALT (U/ml)								
Day 20	8.00	9.00	7.62	7.37	2.20	0.23	0.22	0.29
Day 40	12.87^{a}	11.25^{ab}	10.62^{ab}	9.12^{b}	0.46	0.02	0.00	0.94

AST = aspartate aminotransferase; ALT = alanine aminotransferase.
[1]Treatments: CON = control diet with no additive; SDR 0.5 = control diet with 0.5 g/day of SDR; SDR1 = control diet with 1 g/day of SDR; SDR1.5 = control diet with 1.5 g/day of SDR.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4 *Effect of different levels of SDR[1] on the immune system of calves*

Item	CON	SDR0.5	SDR1	SDR1.5	SEM	Main effect	Contrasts	
							Linear	Quadratic
IL-6 (ng/ml)								
Day 20	1198.8	1142.8	1259.8	1065.6	43.2	0.44	0.46	0.43
Day 40	1247.4^{ab}	1355.3^{a}	1185.0^{ab}	1067.9^{b}	37.7	0.04	0.02	0.11
IL-10 (ng/ml)								
Day 20	479.1	472.3	492.4	482.9	9.9	0.90	0.62	0.94
Day 40	532.0	561.5	491.1	439.9	18.6	0.10	0.03	0.26
INF-γ (ng/ml)								
Day 20	451.5^{b}	448.8^{b}	464.6^{ab}	495.3^{a}	22.0	0.07	0.02	0.22
Day 40	449.3	468.3	460.1	438.6	9.2	0.71	0.64	0.29

IL = interleukin; INF-γ = interferon-γ.
[1]Treatments: CON = control diet with no additive; SDR 0.5 = control diet with 0.5 g/day of SDR; SDR1 = control diet with 1 g/day of SDR; SDR1.5 = control diet with 1.5 g/day of SDR.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

the administration of rumen fluid as an inoculant for pre-ruminant calves have been reported (Pounden and Hibbs, 1949; Muscato *et al.*, 2002). The rumen fluid contains a diversity of microbes, including bacteria, protozoa and fungi, and is a source of microbial metabolites, some proteins, enzymes, amino acids, vitamins, minerals and volatile fatty acids. The present study investigated the potential effects of SDR as a feed additive for suckling dairy calves. Supplementing the starter diet of suckling dairy calves with SDR had no adverse effects on body weight gain and the final weight of calves. Feeding dried rumen fluid had no significant effects on the daily feed intake of calves. Some numerical decrease that was observed on starter intake may be related to the special odour of rumen fluid that remained after spray drying in the final produce. In this study, the rumen-derived feed additive was top-dressed on the starter, and its odour may have some possible effects on the palatability of the feed.

Blood parameters are physiological, pathological and nutritional indices of an animal, which may be affected by nutritional changes and supplements provided in the diet (Alikwe *et al.*, 2010). Among the blood metabolites, the cholesterol concentration was affected by feeding dried rumen fluid to suckling dairy calves. The rumen fluid contains different nutrients, including amino acids; volatile fatty acids such as acetic, propionic and butyric acids; other short-chain fatty acids such as malic acid and salicylic acid; vitamins; and minerals (Elfaki and Abdelatti, 2018). These compounds in dried rumen fluid may stimulate the growth of some useful bacteria in the intestine and, therefore, result in lower blood cholesterol (Alsayadi *et al.*, 2014). The concentrations of liver enzymes, including AST and ALT, decreased with increasing dietary levels of dried rumen fluid. The plasma levels of liver enzymes indicate the integrity of liver tissue and, therefore, general health; so, higher blood concentrations of liver enzymes indicate lower liver tissue integrity. Any inflammation or damage to liver hepatocytes could result in leaking higher-than-normal amounts of liver enzymes into the bloodstream. Higher general health and stronger immune system can ensure lower inflammation and cell injury. The observed lower liver enzymes may be due to an excited immune system on account of feeding SDR, which resulted in higher immunocompetence and, therefore, lower inflammation.

In the present study, the rumen fluid-based feed additive increased blood concentration of INF-γ on day 20 and decreased IL-6 concentration on day 40 of the experiment. T-helper cells (**Th**) are one of the T lymphocyte subsets that are divided into Th1 and Th2 based on their different secretion patterns of cytokines (Carter and Dutton, 1996). Th1 lymphocytes secrete INF-γ that is an essential cytokine for cell-mediated immunity, and Th2 cells produce IL-6 and IL-10, predominantly involved in humoral immunity and allergic responses (Carter and Dutton, 1996; He *et al.*, 2005).

The ruminal fluid has a variety of bacteria and other microorganisms and includes hundreds of bacterial polysaccharides. These bacteria are strong antigens that may stimulate antibody production and remain active after autoclaving (Muscato *et al.*, 2002). It can be speculated that bacterial polysaccharides in rumen fluid are not only stimulating the production of antibodies, they can also, in company with other antigens, stimulate lymphocytes to release cytokines such as IFN-γ. In agreement with our results, Sun *et al.* (2010) reported that feeding a probiotic supplement containing *Bacillus subtilis natto* to suckling dairy calves resulted in more INF-γ production and lower IL-4. Kosaka *et al.* (1998) suggested that the activation of macrophages and natural killer cells might be stimulated by IFN-γ. Muscato *et al.* (2002) fed fresh, autoclaved and centrifuged rumen fluid to suckling calves and observed that calves that received ruminal preparations gained more weight and had fewer scours than untreated calves. They stated that because even autoclaved rumen fluid can have positive effects, it did not act as a probiotic, and the observed effects were attributed to bacterial polysaccharides that act as antigens to stimulate the immune system. The ruminal preparation designed in the present

study, prepared by spray drying, not autoclaving, may contain some live microbes, including bacteria, protozoa and fungi, and, therefore, may have some effects similar to probiotics.

Spray drying is the most common method to remove water content in dairy and pharmaceutical industries (Samborska *et al.*, 2005). In the present study, spray drying was used, along with maltodextrin as an excipient, for dehydration of rumen fluid in order to maintain its residual biological activity, prevent biological degradation, eliminate problems of storage and transport, and eventually prepare a feed additive with special properties for farm animals. Rezai Sarteshnizi *et al.* (2018b) assessed different hydrocolloids, including maltodextrin, sodium alginate, chitosan and guar gum, in two ratios (0.5% or 1% of rumen fluid w/v) as excipient materials for drying rumen fluid by spray drying. They used some enzymatic activities as indices of residual biological activity after drying and reported that drying with 1% maltodextrin resulted in better residual enzyme activities. The biological effectiveness of SDR with 1% maltodextrin was assessed *in vivo* in suckling dairy calves' nutrition. Its useful effects on the immune system of dairy calves show that it can be considered as a new feed additive in animal studies.

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Declaration of interest

There is no conflict of interest for publication of this article.

Ethics statement

The research protocol was approved by the Iranian Council of Animal Care (1995).

Software and data repository resources

The datasets and programs used in the current study are available from the corresponding author on reasonable request.

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