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IMPACT OF FEED SUPPLEMENTATION WITH BALSAM POPLAR BUDS ON PERFORMANCE OF YOUNG BULLS

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Abstract
There is an urgent need to develop new strategies to minimize the environmental impact of animal production and support sustainability of food production and consumption. Feed additives have been for a long time used in animal nutrition to improve animal growth and performance as well as animal health. Balsam poplar plants (Populus balsamifera) is well known as a rich source of bioactive compounds with positive health effects, and might be used in agriculture as a feed additive for ruminants. The aim of the present study was to evaluate the effect of balsam poplar-based additives on growth and performance of fattening young bulls of Simmental breed. In the present study, we used 4 combinations of extract from balsam poplar buds or its components as a feed additives. The animals were given the supplements at the age of 15 months, 3 months before slaughter. The growth and slaughter characteristics of young bulls were studied. After the first and second month of feeding with dietary supplements, animals from the groups fed 10% balsam poplar buds extract and dry shredded balsam poplar buds had significantly higher live weight compared to the control animals fed a diet without any supplements ($P < 0.05$). At slaughter, group fed 10% balsam poplar buds extract had significantly higher live weight compared to control. Average daily gain was also greatest in that group. Major sensory as well as physical and chemical parameters were not affected by balsam poplar-based supplements ($p > 0.05$ for all) and were in line with regulatory meat hygiene requirements.

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Introduction

With continuous growth of world population, the major focus of agricultural sector is to ensure an adequate food supply and food security. Animal health and welfare are closely related to animal production efficiency and has therefore indirectly linked to reduced poverty, which is one of the UN sustainable development goals. Beef cattle is an important agricultural and economic resource in many countries including Kazakhstan. Thus, there is an increasing interest to improve feed conversion and increase growth rate without compromising meat quality and animal welfare. Reduction of negative environmental effects such as carbon emissions and contribute to sustainable land use is also important. Genetic selection, animal management, dietary modification, and application of new feeding techniques and use of feed additives [1–4] can enhance feed conversion and growth rate.

Nutritional needs of livestock animals with respect to energy, protein, minerals and vitamin requirements are well established. To optimize livestock production, antibiotic growth promoters had been used as dietary additive for decades. However, uncontrolled use of antibiotics contributes to increasing resistance in bacteria of human and animal origin [5]. Since 2006, marketing and use of antibiotics as growth promoters in animal production are prohibited in EU although hormonal growth promoters are still in use in US to increase daily weight gain and produce leaner meat [6]. Moreover, consumers prefer "hormone- and antibiotic-free" food because of potential health effects and environmental risks [7]. Over recent decades, much research has also been aimed to improve the quality of feed without use of hormonal growth promoters. This included research on forages and forage conservation [8], as well as the use of various supplements from natural sources [9–11].

There is an increasing interest in using plant bioactive compounds to enhance ruminant health and performance [12]. For this purpose, numerous studies have attempted to use supplements with bioactive compounds or plant extracts [13,14]. Although plant bioactive compounds have lower potency compared to pharmaceuticals, they might affect physiological processes in a positive way and showed numerous benefits for animal health. The existing knowledge on the use of plant bioactive compounds as growth promoters in livestock production was recently reviewed by Valenzuela-Grijalva [15]. Balsam poplar (Populus balsamifera) as a possible feed ingredient for cattle has been studied since they contain a wide range of bioactive compounds with antioxidant activity [16–18]. Cuttings from poplar were shown to have good digestibility [19]. Bark of aspen (*Populus tremuloides*), which belong to the same genus as balsam poplar, was suggested as a good feed for goats [20].

In the North Kazakhstan, there are approximately 1400 ha of balsam poplar plantations but utilization of the buds from balsam poplar is low. Moreover, poplars are early successional species with rapid growth rates. Several studies highlighted balsam poplar buds as an important source of chemically highly diverse bioactive substances such as phenolic compounds and essential oils [18,21]. These compounds are known for their biological functions but are still far from being fully investigated.

We hypothesized that the use of the Balsam poplar buds as a part of animal feed can facilitate digestion process and improve cattle growth and performance. Thus, in the present study we used extract from balsam poplar buds or its components as on growth rate and performance of Simmental bull.

Objects and methods

All procedures used in the study were conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and "The Guide for Care and Use of Laboratory Animals" [22]. During the experiment, all efforts were made to avoid animal suffering.

Animals and feeding

In total, 25 fattening young bulls of Simmental breed, with live weight (LW) from 295 to 307.8 kg and age of approximately 15 months were included into the study. The animals were randomly divided into 5 groups (n=5 in each group). All animals were fed a commercial mixed diet containing wheat grass, hay barley and concentrate. Additionally, each experimental group received a supplement containing extract from balsam poplar buds or its components. The group without any supplement served as a control (group 1). Group 2 received supplement with only polyethylene pellets (once, 500 g /animal). The applied polyethylene pellets are chemically inert substances and do not provide energy. Group 3 received supplement with polyethylene pellets (once, 500 g /animal) in combination with 0.4% essential oil of balsam poplar buds (10 mL once a day for 5 days). Group 4 received supplement with polyethylene pellets (once, 500 g /animal) in combination with a 10% balsam poplar buds extract (10 mL once a day for 5 days). Group 5 received supplement with polyethylene pellets (once, 500 g /animal) in combination with dried shredded balsam poplar buds, containing 2% extract (5 g once a day per 5 days). The animals were given the supplements at the age of 15 months, 3 months (90 days) before slaughter.

Polyethylene pellets in the diet were mixed with a concentrate (1.5 kg of concentrated feed per animal) and fed animals after 18 hour fasting. The animals were slaughtered at a local slaughterhouse using standard procedures.

Measurements after slaughter

Dressing percentage was calculated by dividing the weight of warm carcass by the weight of the live animal and expressed as a percentage. Other measurements of the carcass and veterinary control were performed according to standard methods and veterinary (veterinary-sanitary) rules approved by RK Government No. 7–1/587 of June 29, 2015.

Reaction to the peroxidase (benzidine test) and pH

All laboratory analyses of meat were performed in accordance with the State Standard 23392–78 "Meat. Methods of freshness chemical and microscopic analysis".

Meat extract (2 ml) was added into the test tube, following by addition of 5 drops of 0.2% alcoholic benzidine and 2 drops of 1% hydrogen peroxide. The extract of fresh meat of healthy animals acquired a greenish-blue color, turning into brown in a few minutes. Such coloring is the evidence of peroxidase activity. The meat extract of sick, overworked, dead animals doesn't change the color.

pH of the meat extract was determined by potentiometric method with the use of ionomer 827 pH Lab Metrohm.

Microscopy of smears-marks

The method is based on determining the number of bacteria and degradation of the muscle tissue by microscopy of smears-marks using a binocular microscope Mikmed-5, a mirror digital camera Nikon D5100 with Camera Control Pro2 software and digital cameras Omax A3590U ToupView software. Meat was cut into pieces with a clean scissors from the deep layers of the muscle and attached to a glass slide. Smears were dried, fixed in the flame of the burner and stained as previously described [23].

Sensory test

Sensory tests were performed in accordance with the State Standard 7269–79, which includes evaluation of meat appearance, color and smell, condition of the muscles in the section, as well as the evaluation of broth clarity and flavor.

The appearance and color of the meat samples were evaluated by visual examination. Appearance and color of the muscles in the section were studied in the deep layers of muscle tissue in the fresh meat section. At the same time, there were ascertained presence of adhesiveness and moisture on the section surface of meat by attaching piece of absorbent paper to the section.

The flavor of the surface layer of the test sample was examined by sensory analysis. The sections were made with a clean knife and the flavor in the deep layers was immediately identified.

Evaluation of broth clarity and flavor was performed as follows: 20 g of minced meat were placed into a flask, filled with 60 ml of distilled water and mixed thoroughly. Then, the flask was covered with a watch glass and placed into a boiling water bath. The flavor was examined within the process of heating up to 80–85 °C. To determine clarity, 20 ml of the broth and were transferred into the cylinder and visually adjusted its degree of clarity.

Statistical analysis

All statistical analyses were conducted with Statistical Analysis System, Version 9.4 (SAS Inst., Cary, NC, USA). The mixed model included fixed factor of treatment when evaluating the effect on live weight. Live weight at the beginning of the experiment was included as covariate when evaluating the effect of treatment on other characteristics. Comparisons between the control group fed traditional diet only, and groups with dietary supplements were performed using probability differences. A p-value less than 0.05 was regarded as statistically significant.

Results and discussion

During the experiment span, veterinarians as healthy recognized all animals, and no morbidity and mortality were recorded in any of groups.

At the beginning of the experiment live weight of the young bulls did not significantly differ between the groups (Table 1; $P = 0.974$). After the first and second month of feeding with dietary supplements, animals from the groups 4 and 5 (fed 10% balsam poplar buds extract and dry shredded balsam poplar buds, respectively) had significantly higher live weight compared to the control animals (Table 1, $P < 0.05$). At slaughter, however, the overall effect of group did not reach the significant differences, although according to more specific probability differences test, group 4 fed 10% balsam poplar buds extract had significantly higher live weight compared to control (Table 1). Average daily gain was also greatest in this group.

The determination of the live weight at a given age is probably one of the most obvious and easiest way to access feed efficiency. To the best of our knowledge, this is the first study to investigate the effect of the Balsam poplar buds as a part of animal feed on cattle growth and performance. In the present study, only live weights of the animals in groups 4 and 5 differed from that in control group, indication that the supplements of 10% balsam poplar buds extract and dried shredded balsam poplar buds, containing 2% extract, were most promising in increasing feed efficiency. The loss of statistical significance between group 5 and control group at slaughter time suggests that possible adaptation of the animals to dried shredded balsam poplar buds. In future practical application, the time between providing supplements of balsam poplar-based product and slaughter can be reduced.

Carcass weight was greatest in the groups 2 and 4 (fed with only polyethylene pellets and fed 10% balsam poplar buds extract, respectively) (Table 2). No differences in carcass dressing percentage and other post-slaughter characteristics between treatments was observed ($P > 0.05$ for all, Table 2). Dressing percentage is one of factors defining the value of a livestock animal after slaughter. Numerically,

Table 1. Growth parameters of young bulls

Parameter	Groups					P-value, effect of group
	1 (control)	2	3	4	5	
Live weight before changes in the diet, kg	301 ± 13.6	304 ± 13.6	301 ± 13.6	308 ± 13.6	295 ± 13.6	0.975
Live weight after 1 month, kg	$322^a \pm 3.4$	$326^a \pm 3.4$	$323^a \pm 3.4$	$338^b \pm 3.4$	$334^b \pm 3.4$	0.015
Live weight after 2 month, kg	$341^a \pm 6.0$	$356^a \pm 6.0$	$351^a \pm 6.0$	$366^b \pm 6.1$	$371^b \pm 6.1$	0.017
Live weight at slaughter, kg	$372^a \pm 7.9$	$393^a \pm 7.9$	$387^a \pm 7.9$	$401^b \pm 7.9$	$394^a \pm 7.9$	0.139
Average daily gain, g	$777^a \pm 87.4$	$1017^a \pm 87.5$	$948^a \pm 87.4$	$1103^b \pm 87.4$	$1025^a \pm 88.0$	0.139

Data are presented as least squares (LS) means ± standard errors. LS means with different superscripts within row differs at P < 0.05

Table 2. Effect of dietary supplementation with balsam poplar buds on post-slaughter characteristics

Indicators	Groups					P-value, effect of group
	1 (control)	2	3	4	5	
Carcass, kg	$194^a \pm 5.7$	$211^b \pm 5.7$	$206^a \pm 5.7$	$218^b \pm 5.8$	$209^a \pm 5.8$	0.086
Dressing, %	52.1 ± 1.15	53.7 ± 1.15	53.3 ± 1.15	54.4 ± 1.16	53.0 ± 1.16	0.714
Muscle, %	53.8 ± 0.97	53.3 ± 0.97	53.8 ± 0.97	52.7 ± 0.97	53.7 ± 0.97	0.918
Bones, %	19.2 ± 0.94	17.6 ± 0.94	18.4 ± 0.94	18.9 ± 0.95	18.3 ± 0.95	0.798
Connective tissue,%	11.6 ± 0.61	10.4 ± 0.61	11.0 ± 0.61	11.1 ± 0.61	11.3 ± 0.61	0.741
Fat, %	15.4 ± 0.88	18.6 ± 0.88	16.8 ± 0.88	17.2 ± 0.89	16.8 ± 0.89	0.191

Data are presented as least squares (LS) means ± standard errors. LS means with different superscripts within row differs at P < 0.05 the animals from group 1 (control) had the lowest dressing percentage even though these differences did not reach statistical significance.

The economic value of livestock animals depends on its composition. Nowadays in many countries, a progress was made in reducing the fat content of livestock animals because of consumer demand for lean meat. Generally, the relationship between live weight and fat content is affected by feeding, environmental factors as well as any subclinical diseases that might affect the animal growth rate. In the present study, the animals were kept in the same environment and differed only in feeding strategy. No statistically significant differences between fat content and muscle content were observed suggesting that balsam poplar-based supplements do not exert any effect on carcass composition.

According to organoleptic evaluation and external examination of carcasses and organs of the animals, no deviations were observed in appearance and colour of meat of all groups. Analysis of bacterioscopy of prints smears in meat revealed up to 2.6±0.51 mostly gram-positive coccal microorganisms in the surface of muscle tissue (Table 3). In the deep layers of the samples studied, no microorganisms were found.

The meat pH in all groups corresponded to meat obtained from healthy animals and was in the range from 5.8 ± 0.06 to 6.0 ± 0.05. No primary and secondary protein degradation products in the meat of all groups were observed. The benzidine reaction in all samples was positive.

Thus, major sensory as well as physical and chemical parameters were not negatively affected by balsam poplar-based supplementations ($p > 0.05$ for all).

In the recent years, plant-based bioactive supplements in animal diet received a growing attention as a means to increase production efficiency of ruminants. The phytopreparations from balsam poplar buds contain amino acids, unsaturated fatty acids (malic, tartaric, lemon, linoleic, linolenic, arachidonic), polyphenols (pinostrobin, pinocerbrin, chrysin, tektohrizin, apigenin, kaempferol, quercetin, myricetin, galangin, iszalpinin etc.) carbohydrates, minerals (zinc, manganese, cobalt, copper, iron) and vitamins (A, C, P, E) [16,18,24,25]. Previous studies on the chemical composition of balsam poplar buds also identified the presence of alkanes, terpenes, chalcones and prostaglandins [16,26,27]. This composition might lead to the improvement of fermentation in the rumen, improvement of metabolic processes and suppression of undesirable microflora of the rumen [28]. Additives based on bark from *Populus tremuloides* and leaves and stems from *Populus tremula* were shown have potential to decrease methane production during rumen fermentation [12, 29]. Additionally, many plant-based products represent potential alternative to treat various diseases due to the presence of bioactive compounds with therapeutic properties. Salicin, salicortin, salireposide, and populoside were also identified as bioactive components in balsam poplar responsible for the inhibition of adipogenesis and were suggested as complementary agents in antiobesity and antidiabetic therapies [30]. Currently, the use of the preparations from balsam poplar is limited. In Kazakhstan, balsam poplar buds are used to treat hemorrhoids and scurvy [31]. In Russia, fresh or dry balsam poplar buds are used in forms of an ointment or tincture and used in traditional medicine to treat rheumatism or cold [31]. Development of such preparations represents a complex effort demanding a highly integrated interdisciplinary approach. Recently, antioxidant, anti-inflammatory, hepatoprotective and vasorelaxant activities of *Populus nigra* flower buds ethanolic extract were evaluated using mice as an animal model [32]. It was shown that the extract had anti-inflammatory, hepatoprotective and vasorelaxant properties and no toxic effect was observed after administration of 200 mg/kg during 4 weeks [32].

We believe that our results open new research avenues for the development of novel and safer additives for the improvement of livestock production.

Conclusion

Balsam poplar plants is well known as a rich source of bioactive compounds with positive health effects, and might be used in agriculture to support sustainable animal production and animal health without compromising meat quality and generating environmental impact issues. Animals from the groups fed 10% balsam poplar buds extract and dry shredded balsam poplar buds had significantly higher live weight compared to the control animals fed traditional diet without any supplement ($P < 0.05$). At slaughter, group fed 10% balsam poplar buds extract had significantly higher live weight compared to control.

Table 3. Sensory, physical and chemical examination of meat

Group	Boiling test	Bacterioscopy		Physical and chemical indicators			
		Outer	Inner	Reaction with sulfuric acid copper*	Reaction to peroxidase*	Formaldehyde reaction*	pH
1	clear broth	1.5 ± 0.25	0	–	+	±	6.0 ± 0.07
2	clear broth	2.5 ± 0.25	0	–	+	±	5.8 ± 0.02
3	clear broth	2.0 ± 0.0	0	–	+	±	5.9 ± 0.04
4	clear broth	2.5 ± 0.25	0	–	+	±	6.0 ± 0.05
5	clear broth	2.0 ± 0.5	0	–	+	±	5.8 ± 0.04

** — Negative, + Positive, ± Doubtful*

Average daily gain was also greatest in that group. Major sensory as well as physical and chemical parameters were not affected by balsam poplar-based supplementations ($p > 0.05$ for all) and were in line with regulatory meat hygiene requirements.

Our results indicated that the development of new balsam poplar-based additives for livestock can help to improve animal growth and performance. However, more research is required to establish the optimal dose and period of feeding.

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