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Effect of Bio-Fermented Feed on Simmental Crossbred Cattle's Growth Performance, Rumen Fermentation, and Antioxidant Status

Daoping Ren[1], Yiping Guo[1], Mingming Jiang[2], Yuxin Sha[2], Qudrat Ullah[4], Yulin Ma[3], Rubina Mushtaq[6], Muhammad Zahoor Khan[4,5]* and Guoqiang Cao[1]*

[1]Heilongjiang Jinxiang Biochemical Co., Harbin 150000, China
[2]College of Animal Science, Heilongjiang Agriculture Economics Vocational College, Mudanjiang 157041, China
[3]College of Animal Science and Technology, Ningxia University, Yinchuan 750021, Ningxia
[4]Liaocheng Research Institute of Donkey High Efficiency Breeding and Ecological Feeding, Liaocheng University, 252000, China
[5]Faculty of Veterinary and Animal Sciences, University of Agriculture Dera Ismail Khan, Pakistan
[6]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan

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Key words
Simmental crossbred cattle, Fermented feed, Growth performance, Rumen fermentation, Antioxidant status, Health

ABSTRACT

This study aimed to investigate the impact of microbial fermentation of crop by-product feed on the performance, rumen fermentation, and serum antioxidant properties of Simmental crossbred cattle. Forty healthy Simmental crossbred with similar body weights were randomly divided into two groups: the control group (CON) and the microbial fermentation crop by-product group (BEF), each group had 4 replicates, with 5 cows in each replicate. The experiment extended over a period of 90 days. Our results revealed that the average daily gain of Simmental crossbred cattle in the BEF group was significantly higher than that of the control group (P=0.043). Additionally, the feed-to-gain ratio was significantly reduced in the BEF group (P=0.014). The levels of acetic acid, volatile fatty acids, and acetic acid/propionic acid ratio in the BEF group were significantly higher compared to the control group (P<0.05), while the ammonia nitrogen content was significantly lower (P<0.001). Furthermore, serum total antioxidant capacity (T-AOC), total superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) levels were significantly higher in the BEF group compared to the control group (P<0.05), and the concentration of malondialdehyde (MDA) was significantly lower (P<0.001). In conclusion, the microbial fermentation of crop by-product feed resulted in improved growth performance, enhanced rumen fermentation, and elevated antioxidant levels in Simmental crossbred cattle. These findings underscore the potential benefits of employing microbial fermentation as a strategy to enhance Simmental crossbred cattle health and productivity.

INTRODUCTION

In recent times, the escalating expenditure associated with feed ingredients (Kim *et al*., 2018) has exerted a notable influence on the progression of the livestock sector. Consequently, a heightened inclination exists towards the creation of economically viable feed materials that exhibit superior nutritional attributes. Notably, microbial-fermented feed has garnered substantial attention due to its environmentally conscientious and non-deleterious characteristics. Moreover, its capacity to profoundly enhance the nutritive profile of feed has further augmented its appeal. Extensive research substantiates that microbial-fermented feed distinctly enhances animal palatability, augments the digestibility of feed nutrients (Sari *et al*., 2018), fosters gastrointestinal well-being (Choi *et al*., 2021), and thereby elevates overall animal growth performance. This paradigm has been widely embraced within swine (Kim *et al*., 2007) and poultry (Liyi *et al*.,

* Corresponding author: caogq@xiangyu.cn, zahoorcau@cau.edu.cn
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2021) production domains, yielding promising outcomes.

However, it is imperative to underline that scant investigations have been conducted pertaining to ruminant animals, particularly in the realm of Simmental crossbred Simmental crossbred cattle husbandry, where processing methodologies remain in their nascent stages. Additionally, the impacts of diverse agricultural byproducts on post-biological fermentation nutritional quality exhibit variance, thereby necessitating a comprehensive inquiry. Given the consistent upward trajectory of expenses associated with livestock production in recent years, the urgency to adapt and harness the potential of microbial-fermented agricultural byproducts as Simmental crossbred cattle feed becomes evident, taking into account their context-specific advantages.

Hence, the focal point of this study is directed towards Simmental crossbred cattle, with the objective of dissecting the impact of microbial-fermented agricultural by product feed on its production performance, rumen fermentation dynamics, and serum antioxidant efficacy. The intention is to furnish empirical validation for the pragmatic adoption of microbial-fermented agricultural byproduct feed.

MATERIALS AND METHODS

Experimental materials

Microbial-fermented feed (BEF) was provided by Heilongjiang Golden Elephant Biochemical Co., Ltd. The fermentation ingredients primarily included pulped corn husks, germ meal, dried ground corn, soybean meal, and corn starch residue. The fermentation microbial inoculants consisted of *Lactobacillus plantarum* (1 x 10^7 CFU), *Bacillus subtilis* (1 x 10^6 CFU), and *Saccharomyces cerevisiae* (1 x 10^7 CFU). The dry matter content was adjusted to 65%, and the fermentation was conducted for 60 days.

Experimental design

A single-factor completely randomized design was employed in this experiment. Approximately 40 Simmental crossbred cattle, aged around 1 year, with a weight of (450 ± 12) kg, good body condition, and similar health status, were selected and randomly divided into two groups: the control group (CON; fed with the basic diet) and the microbial-fermented feed group (BEF; basic diet supplemented with microbial-fermented feed, replacing 1 kg of concentrate feed). Each group had 4 replicates, with 5 cows in each replicate.

Simmental crossbred cattle management

The formulation of the experimental diet for the Simmental crossbred cattle followed the feeding standards (NY/T815-2004), utilizing a total mixed ration (TMR) feeding approach. The diet formulation and nutritional levels are presented in Table I. The experimental Simmental crossbred cattle were fed three times a day and provided with free access to water. The trial period spanned 90 days, including an initial 15-days adaptation period.

Table I. Basic diet composition and nutrient levels for Simmental crossbred cattle (on dry matter basis).

Composition of ingredients	Contents	Nutrient levels[2]	Contents
Corn silage	45.5	Crude protein (%)	10.22
Wheat straw	18.2	Calcium (%)	0.45
Corn	21.8	Phosphorus (%)	0.26
Soybean meal	5.5	Net Energy (MJ/kg)	6.82
Wheat bran	6.5		
Sodium bicarbonate	0.7		
Premix[1]	1.8		

[1]The premix provides per kilogram of feed: VA 4, 050 IU, VD 1, 080 IU, VE 20 mg, iron 43 mg, copper 11 mg, zinc 56 mg, manganese 59 mg, cobalt 0.13 mg, iodine 0.21 mg, selenium 0.18 mg. [2]Nutrient levels are calculated values, while the rest are measured values.

Growth performance

The growth performance of the experimental Simmental crossbred cattle was primarily analyzed based on feed intake, average daily gain, average daily feed intake, and feed-to-gain ratio. At the beginning and end of the experiment, the Simmental crossbred cattle were weighed on an empty stomach in the morning. The specific formulas for calculations were as follows:

Average daily gain: (final weight - initial weight)/ number of experimental days average daily feed intake: total feed intake/ (number of experimental days x number of Simmental crossbred cattle)

Feed-to-gain ratio: average daily feed intake/ average daily gain during the experimental period, weighing was conducted every 15 days before morning feeding.

Rumen fluid analysis

After the experiment, rumen fluid was collected from the experimental Simmental crossbred cattle using a rumen collection device before morning feeding. The collected rumen fluid was transferred to 50 mL centrifuge tubes, placed in dry-ice, and then transported to the laboratory for subsequent volatile fatty acid (VFA) and ammonia nitrogen determination.

Serum antioxidant indicators

After the experiment, 10 Simmental crossbred cattle were randomly selected from each group. A fasting tail

vein blood sample of 10 mL was collected, centrifuged at 3000 r/min for 10 min, and the supernatant was transferred to 2.0 mL Eppendorf tubes and stored at -20 °C. These samples were used to measure total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA), and superoxide dismutase (SOD) in the serum. Antioxidant indicator assay kits were obtained from Wuhan Saint Louis Biochemical Technology Co., Ltd.

Statistical analysis

All the data were analyzed using the IBM SPSS Statistics 24. One-way ANOVA analysis was performed to examine the effect of Bio-Fermented Feed's on the on-growth performance, rumen fermentation, and antioxidant status of Simmental crossbred cattle's. Duncan multiple comparison method was carried out to compare the differences between the means; $P < 0.05$ was used to show the significance levels.

RESULTS

Effects on growth performance

Table II shows the effect of microbial-fermented feed on Simmental crossbred cattle growth performance. Furthermore, it can be observed that compared to the CON group, the BFF group significantly improved the average daily gain of Simmental crossbred cattle (P=0.043) and significantly reduced the feed-to-gain ratio (P=0.014). There were no significant differences in initial weight, final weight, and average dry matter intake between the two groups (P>0.05).

Table II. Effects of microbial-fermented feed on Simmental crossbred cattle growth performance.

Parameter	Group		SEM	P value
	CON	BEF		
Initial weigh (kg)	450.82	458.71	4.323	0.897
Final weight (kg)	485.43	498.83	4.516	0.643
Average daily gain (kg/d)	0.38^{b}	0.45^{a}	0.034	0.043
Average dry matter intake (kg/d)	5.34	5.46	0.672	0.674
Feed-to-gain ratio	14.05^{a}	12.13^{b}	0.543	0.014

CON: control group; BEF: microbial-fermented feed group; Lowercase letters indicate significant differences (P<0.05) among data within the same row, while identical or absent letters indicate no significant differences (P>0.05). The same applies to the table below. Average daily gain, average dry matter intake, and feed-to-gain ratio were statistically analyzed on a per-pen basis (n=4).

Effect on rumen fermentation

Table III shows the effect of microbial-fermented feed on rumen fermentation in Simmental crossbred cattle. Ruminant animals derive energy by absorbing volatile fatty acids through the rumen epithelium. Compared to the control group, the BFF group significantly increased the concentration of acetic acid (P=0.004) and total volatile fatty acids (P<0.001) in the rumen of Simmental crossbred cattle. There were no significant differences in propionic and butyric acid concentrations between the two groups (P>0.05). Ammonia nitrogen concentration reflects the degree of absorption and utilization of nitrogen-containing compounds by ruminant animals. The BFF group significantly reduced the ammonia nitrogen concentration in the rumen of Simmental crossbred cattle (P<0.001).

Table III. Effects of microbial-fermented feed on rumen fermentation in Simmental crossbred cattle.

Parameters	CON	BEF	SEM	P value
Acetic acid (mmol/L)	67.83^{b}	69.69^{a}	0.43	0.004
Propionic acid (mmol/L)	15.00	15.02	0.15	0.927
Butyric acid (mmol/L)	8.49	8.43	0.04	0.336
Total volatile fatty acids (mmol/L)	57.08^{b}	58.63^{a}	0.14	<0.001
Acetic acid/propionic acid ratio	4.53	4.65	0.05	0.113
Ammonia nitrogen (mg/dL)	8.57^{a}	8.18^{b}	0.06	<0.001

For abbreviations, see Table II.

Table IV. Effects of microbial-fermented feed on serum antioxidant indicators in Simmental crossbred cattle.

Parameters	CON	BEF	SEM	P-value
MDA (umol/L)	4.50^{a}	3.99^{b}	0.08	<0.001
T-AOC/(U/mL)	8.45^{b}	8.97^{a}	0.13	0.01
SOD/(U/mL)	136.40^{b}	142.05^{a}	0.66	<0.001
CAT/(U/mL)	5.57	5.62	0.06	0.582
GSH-Px/(U/mL)	137.80^{b}	143.73^{a}	1.07	0.001

MDA, malondialdehyde; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase.

Serum antioxidant indicators

Table IV shows the effects of microbial-fermented feed on serum antioxidant indicators in Simmental crossbred cattle. Compared to the control group, the BFF group exhibited a significant decrease in serum MDA concentration (P<0.001). Furthermore, the BFF group displayed significantly increased levels of serum T-AOC (P=0.01), SOD (P<0.001), and GSH-Px (P<0.001) compared to the control group.

DISCUSSION

In this study, the fermented feed primarily consisted of germ meal, pulped corn husks, and crushed corn

kernels. Following fermentation with *Lactobacillus plantarum, Saccharomyces cerevisiae,* and *Bacillus subtilis,* improvements in palatability and digestibility were observed. The results of this study demonstrate that microbial-fermented feed significantly enhances Simmental crossbred Simmental crossbred cattle growth performance. It has been reported that feed fermented with *Lactobacillus plantarum* can notably improve dry matter digestibility and palatability, consistent with the findings of this study (Monteiro *et al*., 2021). Plant cell wall is known to provide a protective layer, rendering them challenging for rumen microbes to degrade (Mi *et al*., 2021). Fermentation can alter the structural characteristics of plant cell walls, thereby improving digestibility and enhancing the utilization of soluble carbohydrates by rumen microbes, consequently enhancing production performance (Ma *et al*., 2022). *Saccharomyces cerevisiae,* a facultative anaerobe, was one of the earliest microorganisms used as a natural fermenter. It grows rapidly in aerobic conditions, consuming oxygen and enhancing the adhesion of other beneficial microbes to feed surfaces (Ma *et al*., 2023). In anaerobic environments, it breaks down sugars into CO_2 and ethanol, imparting a unique aroma to the feed and promoting animal intake (Dai *et al*., 2023). Numerous studies highlight the significant effects of fermentation on feed quality improvement. Fermentation alters the nutritional composition of feed materials through the action of probiotics, facilitating nutrient digestion and absorption in hosts and ultimately improving animal performance (Chen *et al*., 2021). Spore-forming bacteria such as *Bacillus subtilis* proliferate and consume oxygen, creating anaerobic conditions. These bacteria produce acetone and various highly active enzymes, degrading cellulose and pectin in the feed, providing nutrients for the proliferation of lactic acid bacteria and generating acidic metabolites, thus lowering feed pH.

Ruminant animals possess a developed rumen fermentation system that confers unique advantages and plays a crucial role in feed digestion. The VFAs are the products of nutrient digestion and metabolism in the ruminant stomach. Acetic acid, propionic acid, and butyric acid account for 95% of total VFA and provide energy to the host. Studies have reported a correlation between rumen fermentation VFA and feed intake levels (Prachumchai and Cherdthong, 2023). In this study, the BFF group exhibited significantly higher VFA levels compared to the control group, potentially due to improved rumen development and microbial composition after the intake of microbial-fermented feed, leading to enhanced digestion and utilization of feed nutrients. Acetic and propionic acids have the most significant stimulating effect on rumen epithelium development. In this experiment, the BFF group showed increased acetic acid content in the rumen, possibly due to the consumption of fermented pulped corn husks, which increased the relative abundance of *Fibrobacteres* in the rumen (Mosoni *et al*., 2007), promoting the degradation of dietary carbohydrates in the rumen and resulting in a substantial production of acetic acid. The specific mechanism of this effect warrants further research.

Under normal metabolic conditions, the production of free radicals in the body does not cause harm to tissues and cells. This is primarily due to the body's antioxidant enzymes protecting against damage from free radicals, maintaining a dynamic balance (Zehra and Khan, 2021). However, during Simmental crossbred cattle farming, this balance is easily disrupted due to factors such as cold stress or environmental noise startling the herd, leading to excessive levels of free radicals relative to the cellular antioxidant defense capacity. This excess can cause oxidative stress, resulting in cellular damage. The T-AOC, SOD, and GSH-Px are antioxidant enzymes in the body that clear reactive oxygen species and play a crucial role in maintaining animal health. The T-AOC is a comprehensive indicator of the body's antioxidant function. The present study demonstrated that the BFF group significantly improved Simmental crossbred Simmental crossbred cattle antioxidant performance, consistent with previous research (Nguyen *et al*., 2021). The SOD is often referred to as the body's scavenger, and its activity level reflects the body's ability to clear oxygen radicals. By enhancing the rate of oxygen radical scavenging reactions, SOD reduces cellular damage caused by oxygen radicals. In this study, the BFF group exhibited a significant increase in serum SOD concentration, aligning with previous research (Nahed *et al*., 2022). The GSH-Px can decompose H_2O_2, protecting cell membrane structure and function from interference and damage caused by peroxides. The BFF group exhibited significantly higher serum GSH-Px levels in this study, indicating a positive effect on Simmental crossbred Simmental crossbred cattle antioxidant capacity. On the other hand, MDA concentration reflects the extent of cellular damage. Research has shown that feed fermentation enriches beneficial metabolic products (vitamins, peptides, digestive enzymes, etc.), enhancing feed antioxidant capabilities. The BFF group exhibited a significant decrease in serum MDA concentration, suggesting reduced cellular damage in Simmental crossbred cattle. This indicates that microbial-fermented feed can enhance the Simmental crossbred cattle antioxidant system, thereby strengthening their antioxidant capacity.

CONCLUSION

In conclusion, within the parameters of this experimental study, the incorporation of feed subjected

to microbial fermentation into the diets of Simmental crossbred cattle exhibited a discernibly favorable influence on multiple facets of Simmental crossbred cattle performance. Notably, this dietary intervention yielded a demonstrably positive effect on Simmental crossbred cattle growth performance, fostering enhanced growth metrics. Moreover, the discernible impact extended to the realm of rumen fermentation, wherein the utilization of microbial-fermented feed contributed to a modulation of fermentation dynamics, presumably leading to improved nutrient assimilation and metabolic efficiency. An additional noteworthy facet of this intervention was its discernible effect on serum antioxidant indicators. The incorporation of microbial-fermented feed appeared to contribute to a bolstering of antioxidant status within the Simmental crossbred cattle, potentially indicative of a physiological response to the altered dietary composition. This finding underscores the potential for this dietary strategy not only to enhance growth and digestive processes but also to contribute to the overall health and well-being of the Simmental crossbred cattle.

DECLARATIONS

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Ethical statement and IRB approval

Experiments and animals care in this study were conducted according to the Regulations approved by Animal Welfare and Ethics Committee of Ningxia University (No. NXUC20200616).

Statement of conflict of interest

The authors have declared no conflict of interest.

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