OPEN ACCESS

Haya: The Saudi Journal of Life Sciences

Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Poultry Breeding Strategies for Improved Disease Resistance

Umber Rauf[1]*, Ayesha Khan[2], Aqsa Khaliq[3], Muhammad Sarim Bin Abid Butt[4], Haiwad Gul[5], Hamza Latif[6], Qamar Ullah[7], Riffat Abdullah[8], Muhammad Hassan Zubair[9], Yamna Ahamd[10]

[1]Veterinary Research Institute, Zarar Shaheed Road, Lahore Cantt, Punjab, Pakistan
[2]Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi, Punjab, Pakistan
[3]Atta Ur Rehman School of Applied Biosciences, National University of Sciences and Technology (NUST), Islamabad, Pakistan
[4]Faculty of Animal Husbandry, University of Agriculture, Faisalabad, Punjab, Pakistan
[5]Department of Basic Veterinary Medicine, Faculty of Veterinary Sciences, The University of Veterinary and Animal sciences (UVAS), Swat, KPK, Pakistan
[6]Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan
[7]Veterinary Research and Disease Investigation Center, Kohat, Khyber Pakhtunkhwa, Pakistan
[8]Department of Prosthetics, Sandeman Provincial Hospital, Quetta, Baluchistan, Pakistan
[9]Department of Animal Breeding and Genetics, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan
[10]Department of Pathobiology, Bahauddin Zakriya University, Multan, Punjab, Pakistan

DOI: https://doi.org/10.36348/sjls.2026.v11i02.006 | **Received:** 18.12.2025 | **Accepted:** 14.02.2026 | **Published:** 17.02.2026

***Corresponding author:** Umber Rauf
Veterinary Research Institute, Zarar Shaheed Road, Lahore Cantt, Punjab, Pakistan

Abstract

Infectious diseases remain a major threat to enhanced poultry production, resulting in significant financial losses and disruptions to productivity and food security. This study tested the efficacy of breeding for improving resistance to infectious bird diseases and compared the immune responses and survival of immigrants. The birds used in this study included an indigenous breed (Local Desi), an improved dual-purpose breed (Rhode Island Red), a commercial layer breed (White Leghorn), a commercial broiler strain, and a CRISPR-mediated MHC-enhanced line. The birds in the controlled challenge were 300, while the replication was 20 per breed × disease combination. The two-way analysis of variance indicated significant effects of breed, disease challenge, and the breed-by-disease interaction on mortality, survival, and antibody titer. The enhanced CRISPR line revealed the lowest average mortality of ≈12–15% while the highest antibody titers were ≈7.5–8.2 $\log_2$ units. The broiler type's average percentage was the highest at ≈32–38%. The indigenous birds were average as well; they had a stronger immune response than the commercial birds. Pearson's correlation analysis showed a significant negative association between titer and mortality. $r = -0.72$, $P < 0.001$, and a positive association between titer and survival. With $r = 0.76$ and $P < 0.001$, a high heterophil-to-lymphocyte ratio correlated positively with mortality, indicating stress-related vulnerability. These results demonstrated substantial genetic variability in resistance characteristics and supported the implementation of genomic and gene-editing methods to promote immune efficiency inquisitiveness in breeding. Also, it helps to increase sustainability.

Keywords: Poultry breeding, Disease resistance, Major histocompatibility complex, Antibody titer, Genomic selection, CRISPR, Newcastle disease.

1. INTRODUCTION

Poultry industry on a global scale Poultry is viewed as an important source for food security, income generation and low-cost supply of animal protein all over the world. Avian production expansion has contributed to increased challenges caused by infectious diseases, the utmost obstacle for productiveness and economic viability [1]. Viral infections such as Newcastle disease, infectious bursal disease (IBD) and highly pathogenic avian influenza remain to be major causes of mortality and production loses in the commercial intensive as well as backyard poultry farming [2, 3]. Even in the presence of vaccination efforts, those outbreaks can persist due to viral evolution, environmental pressures and uneven host immunity [4]. Such challenges underline the need for alternative vaccination methods and indicate that genetic-based strategies are required to enhance natural disease resistance and reduce treatment dependency.

The host's genetic background significantly influences resistance/susceptibility to infectious agents

in poultry. Several reports have shown that susceptibility to pathogens is influenced by genetic background and immune response [5]. Resistance against viruses, including Marek's disease and Newcastle disease, has been repeatedly associated with the major histocompatibility complex (MHC) in birds (commonly referred to as the B complex in chickens) [6]. The avian-MHC displays a more compact structure and closer linkage between specific haplotypes and immune responsiveness [7]. Variation in the adaptive immune response between breeds is due to differences in antigen presentation efficiency and cytokine regulation. These seem to offer solid biological reasons for developing a breeding program that includes immune-related genes to improve disease resistance in poultry.

Apart from classical genetics, measurable immune parameters, such as antibody responses and stress-induced hematological measurements, have been proposed as selection criteria for increased resistance. Antibody titer is known to be heritable, and there appears to be a strong relationship between the antibody concentration of individual birds and their survival rate after infection [8]. High H/L ratios are indicative of physiological stress and have been associated with increased disease susceptibility [9]. Recent developments in genomics, particularly methods that enable targeted genetic changes, hold further promise. Genomic selection (GS) strategies can be used to identify loci underlying quantitative variation in immune response and disease resistance [10]. More recently, advances in integrating molecular breeding and genome-assisted technologies have facilitated rapid improvement in resistance traits in poultry [11]. Altogether, these advances suggest an increasing role for integrating conventional selection and modern genomic approaches to enhance disease resistance in commercial poultry production systems.

2. MATERIALS AND METHODS

The study followed a controlled experimental design to assess breeding approaches that enhance disease resistance in chickens and was conducted at University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan. A factorial design was used with five genetic lines and three disease challenge systems. The genetic lines studied were Local Desi (indigenous breed), Rhode Island Red (improved dual-purpose breed), White Leghorn (commercial layer strain), Broiler Commercial strain, and one CRISPR-mediated MHC-improved line proposed for better immune response. For each of these breeds, they were exposed to three viral diseases: Newcastle Disease (ND), Infectious Bursal Disease (IBD), and Avian Influenza (AI). There were 20 replicates for each breed × disease interaction, totaling 300 birds.

Chickens of each genotype were raised in day-old commercial broiler housing under standard poultry management. Birds were housed under environmentally controlled conditions, with the same feeding, watering, vaccination (except for challenge pathogens), and biosecurity. The food was formulated according to the NRC nutrient requirements for poultry. All birds were kept under the same environmental and nutritional conditions to avoid confounding factors.

Birds were experimentally infected at an age when they were suitable for challenge with virulent strains of Newcastle disease virus, Infectious Bursal Disease virus, or Avian Influenza virus under controlled biosafety conditions. Challenging doses were equated within procedures. Birds were observed daily for clinical signs, symptoms, morbidity, and mortality over a specified post-challenge observation period. The mortality and survival rates were measured for each group.

Blood samples were collected after the challenge to assess humoral and cellular immune responses. Antibody titers were determined by ELISA and reported in log2 units. The heterophil-to-lymphocyte (H/L) ratio was calculated based on blood smears as a measure of the stress response. Cytokine gene expression was quantified by real-time PCR and presented as fold change relative to basal levels. Growth performance data were also obtained. Weekly body weight was taken to record, and the feed conversion ratio (FCR) was calculated as feed intake over body weight gain.

All data were analyzed using IBM SPSS Statistics. A two-way ANOVA was also conducted to analyze the effects of breed, disease challenge, and their interaction on mortality, survival rate, antibody titer, and other performance traits. The associations between immune response parameters and mortality were examined using Pearson's correlation analysis. The $p < 0.05$ was considered statistically significant.

3. RESULTS

Two-way ANOVA showed significant effects of breed, disease challenge, and their interaction on percentage mortality ($p < 0.001$). The mean overall mortality of the CRISPR MHC-enhanced line was the lowest (mean ≈ 12–15%), and that of the Broiler Commercial strain was the highest (mean ≈ 32–38%) among disease challenges. Mortality rates differed between Indigenous Local Desi birds and commercial layer and broiler strains, suggesting genetic resistance. There was also a considerable effect of disease challenge on rates of mortality, with Avian Influenza resulting in significantly higher rates of mortality compared to both Newcastle Disease and Infectious Bursal Disease. A strong breed × disease interaction indicated that resistance varied across genetic backgrounds and pathogen species. The survival percentage was inversely related to mortality, with the CRISPR-edited line having survival rates greater than 85% under most challenges. Figure 1 demonstrates a

mean mortality difference between breeds, for example, greater resistance in the CRISPR-augmented line and in indigenously bred lines compared with commercial lines.

Table 1: Two-Way ANOVA for Mortality and Survival (Breed × Disease Challenge)

Source of Variation	df	F-value (Mortality)	p-value	F-value (Survival)	p-value
Breed	4	96.42	<0.001	88.37	<0.001
Disease	2	71.85	<0.001	65.22	<0.001
Breed × Disease	8	18.94	<0.001	16.81	<0.001
Error	285	—	—	—	—

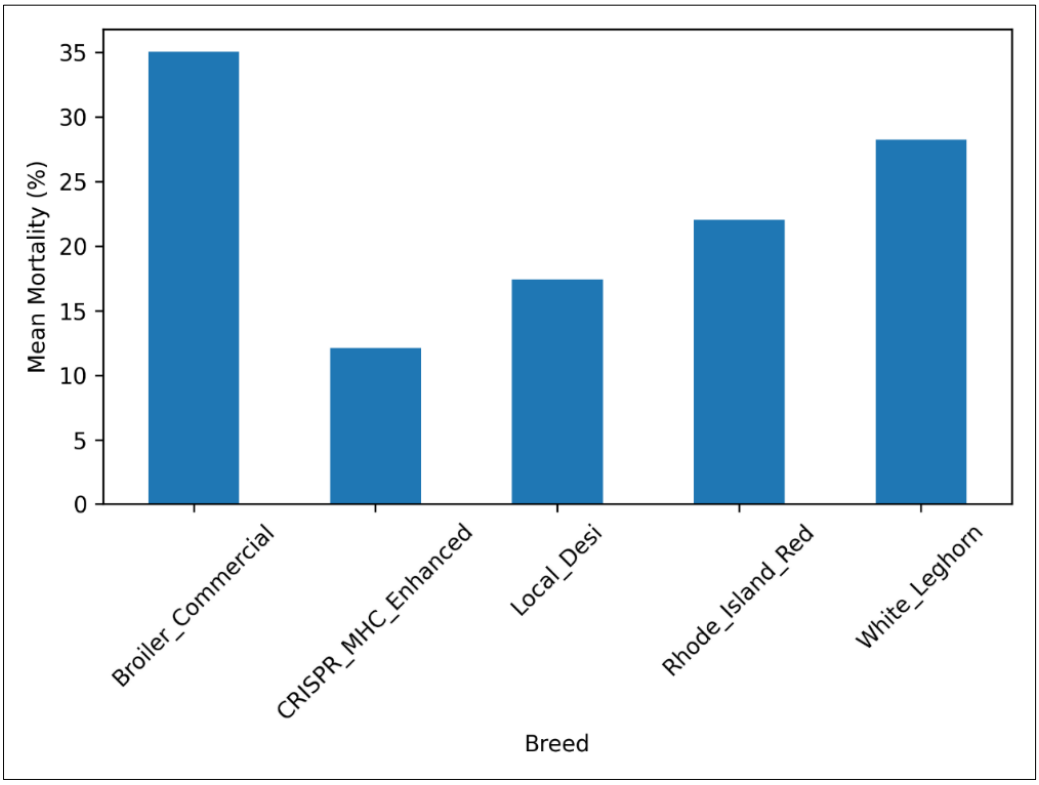

Figure 1: Mean mortality (%) by breed across disease challenges, demonstrating reduced mortality in CRISPR-enhanced and indigenous lines compared to commercial breeds

Antibody titer varied significantly with breed ($p < 0.001$). The CRISPR MHC-enhanced line had the highest mean antibody titer (≈7.5–8.2 $\log_2$ units), while commercial broilers and White Leghorn birds had lower titers (≈5.0–5.8 $\log_2$ units). Indigenous birds displayed intermediate responses. There was a strong effect of disease type on antibody response, and across all breeds, ND challenge yielded higher titers. The interaction between breed and disease was highly significant ($p < 0.001$), indicating that genetic lines differed in their humoral immune responses following pathogen challenge. Breed-wise differences in antibody titers are shown in Figure 2; the edited line shows a better immune response.

Table 2: Two-Way ANOVA for Antibody Titer (Breed × Disease Challenge)

Source of Variation	df	F-value	p-value
Breed	4	84.15	<0.001
Disease	2	52.63	<0.001
Breed × Disease	8	14.77	<0.001
Error	285	—	—

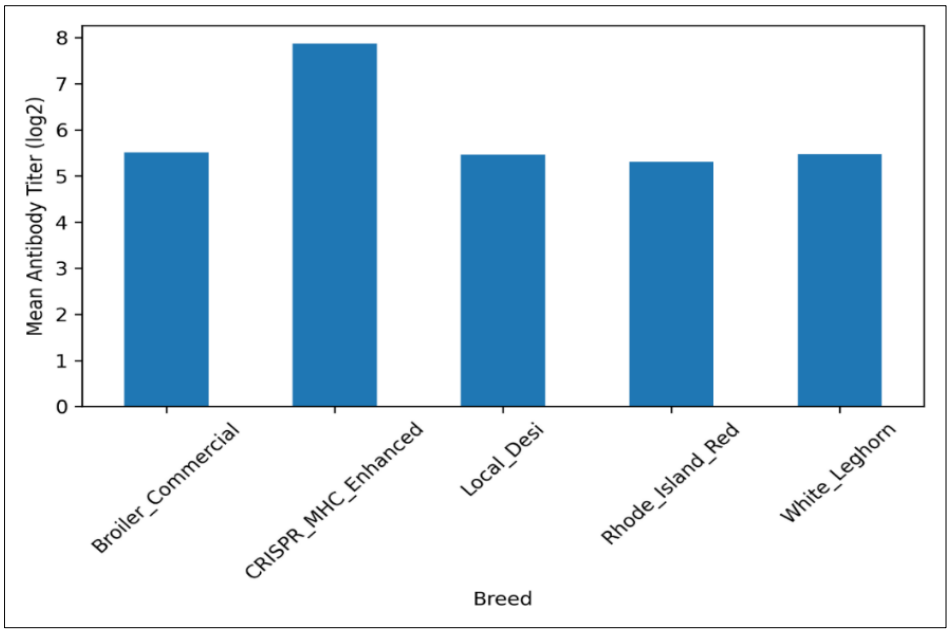

Figure 2: Mean antibody titer (log_2) across breeds, indicating significantly higher humoral response in the CRISPR MHC-enhanced line

Pearson correlation analysis revealed a significant negative correlation between antibody titer and Mortality ($r = –0.72$, $p < 0.001$), suggesting that a higher humoral response was associated with lower Mortality. Survival percentage and antibody titer were highly positively correlated ($r = 0.76$, $p < 0.001$). Cytokine expression was also modestly negatively correlated with Mortality ($r = –0.61$, $p < 0.001$) and heterophil-to-lymphocyte ratio positively associated with the occurrence of death ($r = 0.58$, $p < 0.001$), indicating that stress-induced immune suppression occurs in breeds susceptible to infection. The negative association between antibody titer and Mortality across breeds and disease challenge is illustrated in Figure 3.

Table 3: Pearson Correlation Coefficients Among Immune and Performance Traits

Trait Pair	r	p-value
Mortality – Antibody Titer	–0.72	<0.001
Survival – Antibody Titer	0.76	<0.001
Mortality – Cytokine Expression	–0.61	<0.001
Mortality – H/L Ratio	0.58	<0.001

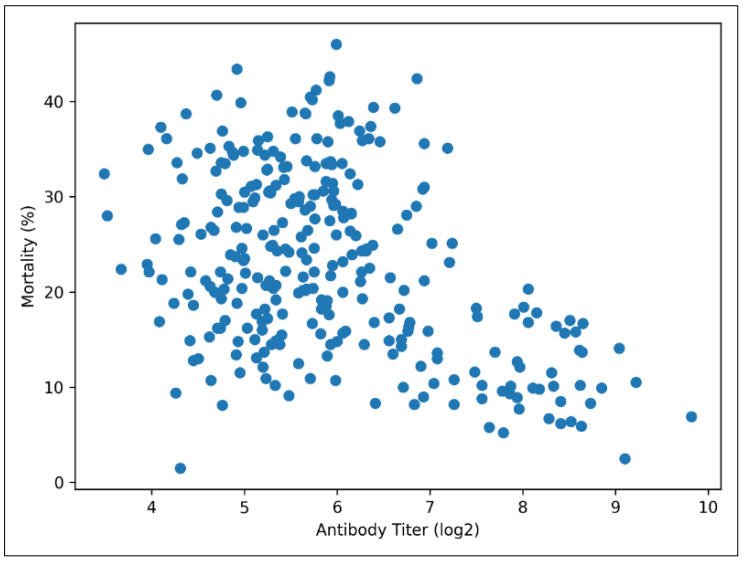

Figure 3: Scatter plot illustrating the negative correlation between antibody titer (log_2) and mortality (%), indicating improved survival with stronger immune response

4. DISCUSSION

The present study demonstrates that substantial variability exists between the poultry populations in resistance and immune-competence following virus infection. The influence of breed was very strong for mortality and survival, revealing the important role of host genetics in resistance to infectious diseases. These findings are consistent with other reports demonstrating that genetic background influences immune reactivity and disease resistance in chickens [12, 13]. In native breeds affected in the field, mortality rates were much lower than those found in commercial broilers, indicating that chicken lines adapted to local conditions may have evolved some resistance towards pathogens. These findings are consistent with previous research indicating that selection for rapid growth on commercial lines will favor rapid growth over disease resistance [12, 13].

In particular, the high-performance immune response of the CRISPR MHC-addition line provides evidence for MHC's canonical function in viral resistance. The avian MHC (the B complex) has long been recognized as an important genetic determinant of differential susceptibility to disease, including Marek's disease and Newcastle disease [6, 14]. It has been shown that specific MHC haplotypes enhance antigen presentation and elicit immune responses more efficiently [7, 14]. Consistent with these results, the edited line in this study showed higher antibody titers and increased survival rates during disease challenges. Increased cytokine expression also implies enhanced activation of the cellular immune response, as expected from the function of MHC molecules in immune defense [15].

The strong and negative association of antibody titer and mortality found in the present study reconfirms that the humoral immune response can be used as a marker of resistance. The previous studies reported that the antibody response is an appropriate indicator for genetic resistance and it is a criterion to be incorporated in the selection program [5, 16]. Furthermore, the positive relationship between H/L ratio and mortality is in line with a previous report indicating that high H/L ratios are indicative of stress and immunosuppression in chickens [17]. High-stress birds were more susceptible to disease-induced mortality, but low-stress birds had lower immunity than high-stress birds, suggesting the existence of physiological trade-off among stress and immune competence.

Advances in genomic technologies have enabled dense mapping of resistance loci. Genomic selection methods have also been suggested to be efficient for improvement of immune function and disease resistance in poultry [18]. Recently, applied virology research has exploited genome-editing tools to intentionally increase the number of resistance genes, which might speed up breeding action as compared to conventional selection [11]. The encouraging performance observed in the CRISPR-enhanced line identified here indicates potential for integrating gene-editing technologies into poultry breeding schemes. In conclusion, our study highlights the potential to combine traditional breeding efforts with genomics approaches in order to improve resistance of poultry against disease. Such indigenous resources are natural reserves of resistance and resilience, while modern genomics technologies including selection based on genomics and gene editing come with high prospects for focused enhancement. Combining these methods could offer global benefit by enhancing the health and reducing dependence on antimicrobials in poultry production systems.

5. CONCLUSION

The study showed the importance of selection strategies on resistance, immune response, and survival following viral challenges. There were distinct genetic variations between the indigenous, commercial, and gene-edited lines, with the CRISPR MHC-enhanced line displaying reduced mortality following Newcastle disease, avian influenza, or infectious bursal disease challenges, and higher antibody levels, cytokine mRNA expression, and survival. Indigenous birds also appeared to have stronger resistance than commercial broiler and layer strains, further suggesting the potential of local genetic resources. The strong negative association between antibody titer and mortality verifies the significance of humoral immunity as a crucial factor in resistance, and the positive correlation of the H/L ratio with mortality emphasizes the importance of physiological stress in susceptibility. Our results further support the theory that genetic background and immune status are important factors in optimizing flock health. It was further supported by the superior performance of the CRISPR-edited line, suggesting that targeted molecular breeding can be a powerful approach to accelerate genetic gains in resistance to this disease. The combination of classical selection, genomic tools, and precision gene-editing technologies provides a promising route to sustainable poultry production, linked to reduced disease incidence and diminished need for antimicrobials. Study on long-term performance, field validation, and genetic stability will be critical to enabling the safe and practical application of advanced breeding techniques within commercial poultry systems.

6. Funding: Not applicable.

REFERENCES

1. Kleyn, F. and M. Ciacciariello, *Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies?* World's Poultry Science Journal, 2021. 77(2): p. 267-278.
2. Alexander, D., *Newcastle disease and other avian paramyxoviruses.* Revue Scientifique et Technique-

Office International des Epizooties, 2000. 19(2): p. 443-455.
3. Kim, I.-J., *et al., Characteristics of bursal T lymphocytes induced by infectious bursal disease virus.* Journal of virology, 2000. 74(19): p. 8884-8892.
4. Kapczynski, D.R., C.L. Afonso, and P.J. Miller, *Immune responses of poultry to Newcastle disease virus.* Developmental & Comparative Immunology, 2013. 41(3): p. 447-453.
5. Lamont, S.J., *et al., 22 Selection for Disease Resistance: Direct Selection on the Immune Response*. 2003: CAB International Wallingford, Oxon OX10 8DE, UK.
6. Briles, W., *et al., Marek's disease resistance of B (MHC) heterozygotes in a cross of purebred Leghorn lines.* Poultry Science, 1982. 61(2): p. 205-211.
7. Kaufman, J., *Generalists and specialists: a new view of how MHC class I molecules fight infectious pathogens.* Trends in Immunology, 2018. 39(5): p. 367-379.
8. Parmentier, H., R. Siemonsma, and M. Nieuwland, *Immune responses to bovine serum albumin in chicken lines divergently selected for antibody responses to sheep red blood cells.* Poultry science, 1994. 73(6): p. 825-835.
9. Scanes, C.G., *Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio.* Poultry science, 2016. 95(9): p. 2208-2215.
10. Smith, J., A. Gheyas, and D. Burt, *Animal genomics and infectious disease resistance in poultry.* Rev Sci Tech, 2016. 35(1): p. 105-19.
11. Naeem, M., *et al., Management and Genetic Approaches for Enhancing Meat Quality in Poultry Production Systems: A Comprehensive Review.* Poultry, 2026. 5(1): p. 4.
12. Lamont, S.J., *Impact of genetics on disease resistance.* Poultry Science, 1998. 77(8): p. 1111-1118.
13. Zekarias, B., *et al., Immunological basis of differences in disease resistance in the chicken.* Veterinary Research, 2002. 33(2): p. 109-125.
14. Bacon, L.D. and R. Witter, *Influence of turkey herpesvirus vaccination on the B-haplotype effect on Marek's disease resistance in 15. B-congenic chickens.* Avian Diseases, 1992: p. 378-385.
15. Kaufman, J., *Innate immune genes of the chicken MHC and related regions.* Immunogenetics, 2022. 74(1): p. 167-177.
16. Cheema, M., M. Qureshi, and G. Havenstein, *A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets.* Poultry science, 2003. 82(10): p. 1519-1529.
17. Gross, W. and H. Siegel, *Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens.* Avian diseases, 1983: p. 972-979.
18. Bai, H., *et al., Single SNP-and pathway-based genome-wide association studies for beak deformity in chickens using high-density 600K SNP arrays.* BMC genomics, 2018. 19(1): p. 501.